

Regional Cerebral Metabolism and Blood Flow During Experimentally Induced Seizures

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This thesis is based on the following papers:

- I. Folbergrová, J., M. Ingvar, and B.K. Siesjö: Metabolic changes in cerebral cortex, hippocampus, and cerebellum during sustained bicuculline-induced seizures. *J. Neurochem.* 1981 37:1228-1238..... 33
- II. Siesjö, B.K., Ingvar, M., and Westerberg, E.: The influence of bicuculline-induced seizures on free fatty acid concentrations in cerebral cortex, hippocampus and cerebellum. *J Neurochem.* 1982 39:796-802..... 45

- III. Ingvar, M., Lindvall, O., Folbergrová, J., and Siesjö, B.K.: Influence of Lesions of the Noradrenergic Locus Coeruleus System on the Cerebral Metabolic Response to Bicuculline-Induced Seizures. *Brain Res.* in press.... 53
- VI. Ingvar, M., Abdul-Rahman, A., and Siesjö, B.K.: Local cerebral glucose consumption in the artificially ventilated rat: Influence of nitrous oxide analgesia and of phenobarbital anaesthesia. *Acta Physiol Scand* 109:177-185, 1980..... 67
- V. Ingvar, M., and Siesjö, B.K.: The effect of nitrous oxide on local glucose utilization in awake minimally restrained rats. *J Cereb Blood Flow Metabol* in press.... 77
- VI. Ingvar, M., Nilsson, B., and Siesjö, B. K.: Local Cerebral blood flow in the brain during bicuculline induced seizures and the modulating influence of inhibition of prostaglandin synthesis. *Acta Physiol Scand* 111:205-212, 1981..... 89
- VII. Ingvar, M., and Siesjö, B.K.: Local blood flow and glucose consumption in the rat brain during sustained Bicuculline-induced seizures. *Subm for publ*..... 97



Introduction

For many years, changes in brain metabolism and circulation during experimentally induced epileptic seizures have been receiving keen attention. One reason for this is that experimental seizures, with their dramatic effects on neuronal activity, offer good models for studies of the coupling between neuronal function, metabolism, and blood flow. Experiments with different seizure inducing agents give valuable information on the cellular mechanisms that lead to seizure activity as well as those that determine the seizure propagation within the brain. Finally, experimental seizures are invaluable for the exploration of factors that could explain why prolonged and/or recurrent seizures induce neuronal cell damage in the brain, and why such damage tends to be localized to certain selectively vulnerable regions.

This thesis concerns cerebral metabolic and circulatory changes in the rat brain during prolonged seizures induced by bicuculline, a plant alkaloid which has the ability to block GABA receptors in the brain (Curtis et al 1974). In the experiments to be described, methods were utilized that allow studies of **regional** or **local** changes in brain metabolism and circulation. They give information, therefore, on metabolic and circulatory events that follow upon a generalized dysinhibition of neuronal cell populations in the brain as well as on events that could help to explain the phenomenon of selective neuronal vulnerability.

Background

1. Brain metabolism and circulation.

Under normal conditions glucose serves as the only substrate for the adult brain. The high energy demands of the brain cells are met by complete oxidation of glucose to carbon dioxide and water, a chain of reactions that will yield over 30 moles of ATP for each mole of glucose consumed. All cellular work, whether it involves repumping of ions (e.g. Na^+ , K^+ , and Ca^{2+}) following depolarization of membranes, intracellular transport of macromolecules and whole cell organelles, or synthesis, leads to a breakdown of ATP to ADP and inorganic phosphate (P_i). Any decrease of ATP and increase in ADP will, by the adenylate kinase reaction, lead to an accumulation of AMP, which, in turn, is further metabolised to a series of degradation products, including adenosine. The balance between the concentrations

of the different adenine nucleotides is regulated within very narrow limits. Thus, the adenylate energy charge (EC), an expression coined by Atkinson (1968), as

$$EC = \frac{[ATP] + 0.5 [ADP]}{[ATP] + [ADP] + [AMP]} \quad (1)$$

is normally regulated to a constant value of about 0.94, and it is only in severe situations such as those caused by severe oxygen or glucose deficiency that marked derangements are seen. It should be recalled, though, that metabolic stress (e.g. tissue hypoxia) of a degree that does not measurably perturb the energy charge of the tissue may well be accompanied by activation of anaerobic glycolysis with an ensuing accumulation of lactic acid.

Breakdown of ATP as a result of neuronal depolarization is initiated by the transmembrane fluxes of Na^+ and K^+ which activate the membrane bound ATPase. However, several other reactions occur which lead to ATP utilization. These include activity coupled influx of Ca^{2+} into the nerve cells, as well as reactions that lead to turnover of membrane constituents. All cell membranes contain phospholipids. It is now recognized that some of these phospholipids have a rapid turnover, and that cell activity leads to lipolysis with production of lysophospholipids and free fatty acids (FFA), among them arachidonic acid. Since the latter is the precursor of, among other compounds, prostaglandins, one can envisage that depolarization of cells, or interaction between agonists and receptors, gives an increased phospholipid turnover and production of FFA and prostaglandins (see Hawthorne and Pickard 1977, Hirata and Axelrod 1980). The lysophospholipids and FFA formed can, if they accumulate to high concentrations, induce adverse cellular reactions; e.g. polyenoic FFAs have been shown to precipitate cellular edema (Chan and Fishman 1978). It is to be expected, therefore, that the concentrations of FFAs are kept at low levels by efficient reacylation processes. Consequently, any rise in FFA concentration by excessive production or deficient reacylation (e.g. due to ATP shortage) represents a disturbance of normal cell metabolism with possible pathological consequences.

Several comprehensive reviews have been issued over the last 25 years on the regulation of the cerebral circulation and metabolic rate, and the most recent of these have extended the analysis to the

local tissue level (e.g. Lassen 1959, Betz 1972, Olesen 1974, Siesjö 1978, and Sokoloff 1981). Only a very brief overview will be given here with an emphasis on aspects of the metabolism/blood flow couple that are relevant to the subject of the present thesis.

The blood flow to the brain is autoregulated, i.e. (within certain limits) it does not change with fluctuations in arterial blood pressure. The autoregulation together with the normal coupling of metabolic rate and blood flow, provide the brain with purposeful homeostatic mechanisms. Other mechanisms of importance are those that lead to brisk vascular responses in hypercapnia and hypoxia. Around carbon dioxide tensions of 40 mmHg the blood flow to the brain increases 4-5% for each mmHg increase in PCO_2 . In the normal situation oxygen tension does not play the same role, and it is only when the PO_2 falls to low levels (below 50 mmHg) that hypoxia leads to a marked vasodilatation.

The mechanisms that adjust blood flow to the metabolic demands are not accurately known. Probably, many metabolic effects of cell activity contribute to CBF regulation since it has been found that vasodilatation occurs in response to increased extracellular concentrations of H^+ , K^+ , and adenosine, and to reduced concentrations of Ca^{2+} (see also Winn et al 1981). Recently the cyclo-oxygenase product prostacyclin has been implicated in the control of CBF. The evidence is based in the fact that at least in monkeys, rats and man, the cyclooxygenase inhibitor indomethacin reduces resting CBF and markedly attenuates the CO_2 responsiveness of the vessels (Pickard and Mackenzie 1973, Sakabe and Siesjö 1979, Wennmalm et al 1981). However, the drug does not influence the vascular response to hypoxia (Sakabe and Siesjö 1979).

2. Cerebral changes during seizures

A seizure is defined as "the sudden onset of an intense, rapidly repetitive focal or generalized electrical discharge from the brain" (Duffy and Plum 1981). As a result seizures place the largest known metabolic load on the brain. Depending on the species used, the type of seizures studied, and the method employed, different investigators have found an increase in metabolic rate from 1.5 to 5 times the normal (Schmidt et al 1945, Collins et al 1970, Duffy et al 1975, Borgström et al 1976, Meldrum and Nilsson 1976). Probably, true metabolic rate during seizure discharge does not exceed 300% of

control. We recognize that published estimates of metabolic rate during bicuculline induced seizures, pertaining to the whole brain or cerebral cortex (Borgström et al 1976, Meldrum and Nilsson 1976), demonstrate a 2- to 3-fold increase in oxygen and glucose consumption, and recall that this increase is sustained for at least two hours unless restriction of the glucose or oxygen supply leads to attenuation of the seizure discharge (Blennow et al 1979).

It was an early discovery that seizures are accompanied by hyperemia, and Roy and Sherrington (1890) concluded that products of the cerebral metabolism are responsible for the local coupling of cerebral blood flow and metabolism. Among the results that led to this conclusion was the observation that the blood flow in the brain markedly rose if strychnine was injected i.v. into dogs. With the marked intracerebral vasodilation, autoregulation is abolished during seizure activity (Plum et al 1968). The blood pressure therefore has great bearing on what magnitude the CBF increase will reach. Reports on whole brain and regional increases in CBF during seizures vary between 1.5 and 9 times that of control (Jasper and Erickson 1941, Plum et al 1968, Brodersen et al 1973, Howse et al 1974, Meldrum and Nilsson 1976). Several investigators have reported that at least at the beginning of a seizure period the CBF increase matches the increase in the metabolic rate (Gurdjian et al 1947, Meldrum and Nilsson 1976, Plum et al 1968, Caspers and Speckman 1972). However, the question has remained whether prolonged or recurrent seizures are accompanied by a secondary reduction in blood flow, creating a mismatch between metabolic rate and circulation.

There is now extensive information on seizure-induced metabolic changes in cerebral tissues. If the experimental animal is given supported ventilation, and if systemic complications such as hypoxia, hypotension, hypoglycemia and hyperthermia are avoided, then there are only moderate changes in the EC in the cerebral cortex. The EC may be upheld to values exceeding 95% of control in spite of an intense seizure discharge (Duffy et al 1975, Chapman et al 1977, Blennow et al 1977, 1979). However, the lactate concentration is increased from about 1.5 $\mu\text{mol/g}$ to 10 $\mu\text{mol/g}$ and maintained at that level for the duration of the seizure activity. The lactate pyruvate ratio is raised 3- to 4-fold (Duffy et al 1975, Chapman et al 1977). The cellular glucose concentration falls within the first minutes of a seizure period by

one third in ventilated animals but, unless the blood glucose falls below 10 $\mu\text{mol/ml}$, the tissue values remain at 1 $\mu\text{mol/g}$, or more (Duffy et al 1975, Chapman et al 1977; see Blennow et al 1979 for values obtained in animals developing hypoglycemia during seizures).

The metabolic perturbations observed during seizures encompass changes affecting neurotransmitter systems and other compounds considered to modulate neuronal activity or CBF. For example, seizures are accompanied by changes in the rates of synthesis and tissue levels of monoamines suggesting that the central noradrenergic neurons show a grossly enhanced activity, and that the activity of the dopaminergic neurons decreases (Calderini et al 1978). Furthermore, the initial decrease in ATP concentration is associated with increased levels of adenosine (Schrader et al 1980, Winn et al 1979), and with accumulation of the cyclic nucleotides cAMP and cGMP (e.g. Sattin 1971, Ferrendelli et al 1976). Finally, changes occur suggesting an increased turnover of membrane bound phospholipids. Since the free fatty acid pool in the brain was characterized (Lunt and Rowe 1968) it has repeatedly been shown that the pool increases during ischemia (Bazán 1970a, 1976), and recent results show that it increases in severe hypoglycemia as well (Agardh et al 1980). There is evidence that at least during ischemia the arachidonic acid is derived from phosphatidylinositol and phosphatidylcholine (Marion and Wolfe 1979). Early as well as more recent results demonstrate that seizures induced by electroshock or metrazol in spontaneously breathing animals are accompanied by a similar accumulation of FFA (Bazán et al 1970b, Marion and Wolfe 1978). Quite recently it has been shown that such an accumulation occurs during bicuculline-induced seizures even if controlled ventilation secures an adequate cerebral oxygen supply (Chapman et al 1980).

Several of the metabolic changes observed during seizures provide putative coupling factors that could explain the increase in CBF. Thus, apart from the fact that seizures are accompanied by release of K^+ and uptake of Ca^{2+} (Heineman et al 1977), production of H^+ and adenosine occur, and the conditions are at hand for an arachidonic acid cascade, including the release of prostaglandins.

Histopathologically, the brain is often considered to have areas that are selectively vulnerable, i.e. areas that are prone to develop cell damage after serious but sublethal insults such as

transient global ischemia, or hypoglycemia. Such areas include the neocortex, the hippocampus, the caudoputamen, and the cerebellum. All these areas have also been found to be prone to develop neuronal cell damage after repeated or sustained epileptic seizures in patients (for review, see Corsellis and Meldrum 1976). Experimental work has confirmed this but shown that some differences exist (Meldrum and Brierly 1973, Meldrum et al 1974, Blennow et al 1978, Söderfeldt et al 1981). Thus, if animals with status epilepticus are ventilated and systemic complications avoided, the histopathological outcome is better, and the Purkinje cells in the cerebellum are spared (Meldrum et al 1973, Blennow et al 1978). In other words, under controlled experimental conditions the cerebral cortex and the hippocampus represent "vulnerable" structures, and the cerebellum a "resistant" one.

Aim of the present study

With the background that has been summarized above we wanted to evaluate changes in regional cerebral energy metabolism and blood flow during prolonged status epilepticus and thereby explore possible relationships between regional changes in the metabolism/blood flow couple and the reported regional differences in the structural alterations.

In the first part of the study regional differences in cerebral energy metabolites and free fatty acids were evaluated in the cerebral cortex, hippocampus, and cerebellum. For reasons given below, the influence on these parameters exerted by the nucleus of the locus coeruleus during status epilepticus was also evaluated.

In the second part, emphasis was laid on evaluation of the validity of the autoradiographic technique for studying local glucose utilization. The evaluation was extended by measurements on minimally restrained animals and by an estimation of the role played by nitrous oxide.

In the last part of the study local blood flow and glucose utilization were examined during status epilepticus with autoradiographic techniques, with the purpose of exploring whether selectively vulnerable regions differ from others in terms of changes in metabolic rate, or in the metabolic rate/blood flow couple.

Methods

Animals and operative techniques.

All the experiments were carried out on male SPF Wistar rats (Møllegaards Avslaboratorium, Copenhagen), that had free access to food (Astra-Ewos, Södertälje) and water until the time of the experiment.

All animal preparations, except the stereotaxic lesions of the locus coeruleus, were performed under halothane and nitrous oxide/oxygen (7/3) anesthesia. The animals were immobilized with d-tubocurarine and ventilated with a volume respirator (Braun, Melsungen). The respirator was set to yield a PCO_2 between 30 and 40 mmHg and the arterial PO_2 was kept over 90 mmHg. Body temperature was kept close to 37°C. In all but the unparalyzed animals (V) EEG was recorded. Arteries were cannulated to obtain blood pressure recording and blood samples for analysis of blood gases, pH, and glucose. The brachial artery was chosen as the sampling artery in the blood flow experiments (Dahlgren et al 1981). The femoral veins or one of the tail veins were also cannulated. These lines were used for infusion of drugs, blood, and isotopes.

In animals used for analysis of cerebral metabolites, a skin incision was made over the skull bone to accommodate a plastic funnel for later freezing of the brain *in situ* (I,II,III,VII). Animals used for measurements of local cerebral blood flow were placed in a guillotine for simultaneous decapitation with the last arterial sample (VI,VII). When measurement of regional blood flow was done a burrhole over the caudal part of the superior sagittal sinus was drilled in order to obtain venous blood representative for cerebral venous outflow.

Sampling of tissue and analytical techniques

Blood gases and pH: PO_2 , PCO_2 , and pH in arterial blood were measured in anaerobically obtained samples of 150-200 μ l, using microelectrodes working at 37 °C with due correction for any deviation from this temperature in the animal.

Cerebral metabolites: At the end of the experimental period the animals were frozen *in situ* with liquid nitrogen (Pontén et al 1973). The brain was then chiseled out and stored at -80 °C for later

dissection in -20 to -22 °C. The samples were then either extracted directly or restored in -80 °C for later extraction. Details on the dissecting procedure are found in paper I under Methods.

Carbohydrate substrates and labile phosphates: Analytical procedures for the carbohydrate substrates and labile phosphates are based on conventional fluorometric techniques (Lowry and Passoneau 1972), with the analytical conditions described previously (Folbergrová et al 1972 a,b). Blood glucose was measured with a similar technique based on the hexokinase reaction. Plasma glucose was either measured in a Beckman automatic glucose analyzer or with the same technique as for whole blood.

Fatty acids: Tissue extractions were made in chloroform and methanol and the FFA band separated by thin layer chromatography. The free fatty acids were esterified, and the methylesters were measured with gas chromatography using an internal standard, as described previously (Rehncrona et al 1981).

Cyclic nucleotides: Cyclic AMP was measured with a protein binding technique using the Cyclic AMP Assay kit purchased from Amersham, England. Cyclic GMP was determined according to Steiner et al (1972) the kit being supplied from New England Nuclear, Boston, USA.

Noradrenalin: NA was determined in tissue as described by Schmidt et al (1981) using a radioenzymatic technique based on the work of da Prada and Zürcher (1976).

Measurement of cerebral blood flow and glucose utilization

Regional cerebral blood flow: Regional-CBF was determined with a modification of the Kety and Schmidt technique (1948), as described by Norberg and Siesjö (1974) and Berntman et al (1979). The animal was allowed to equilibrate with a ^{133}Xe -containing oxygen/nitrous oxide mixture, and then the washout of ^{133}Xe from the brain was studied. The brachial vessel was used for sampling of arterial blood, and the venous blood was obtained from the superior sagittal sinus. Steady state in flow during measurement was assumed if four matched arterio-venous oxygen difference measurements agreed within 10%. The total oxygen contents of the arterial and venous microsamples were measured according to Borgström et al (1974).

Local cerebral blood flow: Landau et al (1955) described the autoradiographic technique for measurement of l-CBF. The tracer used by these authors was impractical as it was a gas ($\text{CF}_3\text{I}^{131}$). We adopted

the technique of Sakurada et al (1978), using ^{14}C -iodoantipyrine instead as the diffusible tracer. The tracer was infused intravenously over a period of 45s, or less (see Eklöf et al 1973). During the infusion repeated, timed arterial samples were taken. Simultaneously with the last sample the animal was decapitated. The brain was then removed from the skull and frozen. The ^{14}C -activity of the blood samples was determined with β -scintillation counting.

Local cerebral glucose consumption: We used the technique described by Sokoloff et al (1977). At the beginning of a 45 min period ^{14}C -deoxyglucose was infused over a period of 30 s intravenously. During the 45 min several timed blood samples were taken for later analysis of their glucose content and ^{14}C -activity. The ^{14}C activity of the brain was then determined autoradiographically.

General procedures for autoradiography: After the brain was removed from the skull it was frozen in isopentane chilled to -50 to -70 $^{\circ}\text{C}$. It was then stored at -80°C until sectioning. The sections were prepared using a Leitz sledge microtome calibrated to 20 μm . The sections used were dried on a hot plate and then exposed an X-ray film for one week, together with a set of calibrated ^{14}C standards. The staining of the film was evaluated manually using a densitometer (Macbeth TC504 or TD501) with an aperture of 1 mm. Each structure was measured bilaterally on at least three different sections, and the normal number of readings was approximately 12 per structure in each rat.

Calculations

The energy charge of the adenylate pool was calculated according to Atkinson (1968)

The cerebral metabolic rate for oxygen was calculated as the product of the cerebral blood flow and difference in arterial and venous oxygen content.

Local blood flow was calculated on a microcomputer with a program using an iterative procedure. The equation used was that from Sakurada et al (1978):

$$C_1(T) = \lambda \cdot K_1 \int_0^T C_a \cdot e^{-K_1(T-t)} dt \quad (2)$$

where $C_1(T)$ is the tissue concentration at the time (T) after the start of the infusion of the tracer (^{14}C -iodoantipyrine) into the

animal, λ is the tissue/blood partition coefficient, C_a is the arterial concentration of the tracer, and K_1 is defined as

$$K_1 = mF/\lambda W \quad (3)$$

here F/W denotes flow per unit mass and m is a factor between 0 and 1 expressing any diffusion limitation of the tracer used. In the present experiments we have assumed this factor to be 1, i.e. that iodo-antipyrine reaches diffusion equilibrium infinitely fast.

Local cerebral glucose utilization was calculated according to Sokoloff et al (1977).

$$R_i = \frac{C_i^*(T) - k_i^* e^{-(k_2^* + k_3^*)T} \int_0^T C_p^* e^{(k_2^* + k_3^*)t} dt}{\left[\frac{\lambda \cdot V_m^* \cdot K_m}{\Phi \cdot V_m \cdot K_m^*} \right] \left[\int_0^T \left(\frac{C_p^*}{C_p} \right) dt - e^{-(k_2^* + k_3^*)T} \int_0^T \left(\frac{C_p^*}{C_p} \right) e^{(k_2^* + k_3^*)t} dt \right]}$$

Here, R_i stands for glucose utilization per unit mass of tissue; C_p and C_p^* are the arterial plasma concentrations of glucose and deoxyglucose respectively; T is the time of decapitation after the infusion of the radioactive deoxyglucose; $C_i^*(T)$ is the amount of radioactivity in the tissue at the end of the experiment; k_i^* is the rate constant for deoxyglucose entry into the precursor pool (defined as intracellular unphosphorylated deoxyglucose). k_2^* and k_3^* are the rate constants for transport of deoxyglucose from the precursor pool to the plasma and the phosphorylation of deoxyglucose, respectively. λ is the ratio of distribution volumes of the two hexoses and Φ equals the fraction of glucose-6-phosphate that enters the glycolytic pathway during steady state, while K_m^* , V_m^* , K_m , and V_m are the Michaelis-Menten constants and maximal phosphorylation rates for deoxyglucose and glucose, respectively.

The rate constants for glucose and deoxyglucose used are those given by Sokoloff et al (1977). The lumped constant (the constant that among other things corrects for the fact that deoxyglucose is used as a tracer instead of glucose) was assigned the value of 0.48. In the last paper a special procedure was adopted for recalculation of the lumped constant as there is a change in the lumped constant when the

plasma to brain glucose ratio is changed (see paper VII for discussion)

Evaluation of statistical differences were performed using the Student's t-test for paired or unpaired data or the Mann-Whitney U-test as specified in each paper.

RESULTS AND DISCUSSION

In all studies in which bicuculline induced seizures were employed, the seizure rats had an ictal increase in blood pressure after the injection of bicuculline. The arterial PCO_2 fell markedly in the beginning but normalized after about one hour. All seizure animals became markedly acidotic, with a blood pH close to 7.0.

Paper I.

In order to obtain more information on the biochemical mechanisms underlying epileptic cell damage we analyzed three different cerebral regions in a rat model of status epilepticus known to cause cellular alterations after 1 to 2 h of seizure activity (Blennow et al 1978, Söderfeldt et al 1981). Tissue was sampled for analysis 20 min and 2 h after the injection of bicuculline. The primary objective was to see whether the energy state was more perturbed in the vulnerable regions (parietal cortex and hippocampus) than in the resistant one studied (cerebellum). As there were signs that the perturbation of the energy state was more severe in the vulnerable regions at 2 h due to substrate depletion, we also studied animals receiving an infusion of glucose i.v. during the last hour of the experiment.

After 20 min of seizures some decrease in ATP content was apparent both in the vulnerable regions (cortex and hippocampus) and in the resistant one (cerebellum). At 2 h there was an indication that the glucose supply had become limiting for the ability of the cortex and the hippocampus to maintain their energy state as there was an inverse correlation between the tissue glucose concentration and the perturbation of the energy state. The animals also had a secondary decrease in lactate with a persistently low EC and no glycogen resynthesized which further points at the limitation of the glucose supply. In the animals receiving an infusion of glucose there was still a small decrease of the EC at 2 h both in the hippocampus and in the parietal cortex; it was however much less marked in spite of a maintained increased tissue lactate. The cerebellum in the animals without glucose infusion had at 2 h normalized its ATP after a decrease

seen at 20 min. The cerebellum had higher glucose content than the cortex and hippocampus at 2 h of seizure activity and showed a nearly complete resynthesis of glycogen and almost normalized the lactate content at 2 h.

The small but persisting energy failure present in the cortex and the hippocampus but not in the cerebellum at 2 h makes it tempting to conclude that the energy failure in itself may contribute to the cell damage. However, the increase in metabolic rate seen in the cortex and in the hippocampus but not in the cerebellum (VII) makes it just as likely that the perturbed energy charge merely reflects the increased metabolic rate.

The levels of cyclic AMP and cyclic GMP have repeatedly been shown to increase during seizure activity (Sattin 1971, Folbergrová 1977, Lust et al 1975, Ferrendelli and Kinscherf 1977). The question has remained though, whether these increases occur during seizures in animals with a PO_2 not influenced by asphyxia. At present it is not possible to offer a straightforward explanation for these increases, but there is evidence suggesting that the changes in the cAMP and cGMP to some extent reflect the balance between inhibition and excitation in the tissue (Stone et al 1975). Activity in central noradrenergic pathways and cAMP both inhibit firing rates in many nerve cells, and stimulation of the locus coeruleus increases the cAMP content in the forebrain (Korf and Sebens 1979). Increase in tissue adenosine content also leads to an increase in cAMP concentration (Huang et al 1973). In other words, the increased turnover in the NA-system in the brain during status epilepticus (Calderini et al 1978) together with the small perturbation of the energy state makes an increase in cAMP expected during status epilepticus. The cGMP system has been shown to be activated by a number of agents such as Ca^{2+} (Ferrendelli et al 1976), convulsive and subconvulsive doses of several excitatory drugs (Opmeer et al 1976), and different neurotransmitters (Berridge 1979).

The changes in cAMP were only significant in the cerebral cortex, and in this structure the cGMP was increased threefold during the two hour period. The hippocampus, where the changes in most other variables measured were similar to cortex, did not display any difference from control in the levels of cyclic nucleotides. However, in this structure there was a large variation in the levels of cGMP

obtained in the seizure animals. In the cerebellum the cAMP system seemed to be unaffected at 20 min and 2 h after the seizure induction whereas the increases in cGMP were massive. The imbalance between the cyclic nucleotides in the cortex and in the hippocampus may reflect the imbalance between excitation and inhibition. However, as the cerebellum displayed the largest imbalance in spite of its low epileptic activity (VII), the interpretation of the data must await further results (cf Siesjö et al 1982).

Paper II

The accumulation of free fatty acids was measured in ventilated animals in order to explore the time course in changes during long-standing seizures without complicating factors like hypoxia or hypotension. The analysis was carried out regionally to obtain information whether the cerebellum (a resistant structure) showed a similar pattern in the changes of the levels in the free fatty acids as in the cerebral cortex and hippocampus. In order to explore whether formation of prostaglandins was a major reason for the secondary decrease of the arachidonic acid, observed during seizures, one group of rats was treated with indomethacin, a cyclooxygenase inhibitor.

The concentrations of FFA increased precipitously already after 1 min and then remained essentially unchanged at a level of 2.5 times control in the cerebral cortex for twenty min. The concentrations subsequently fell but remained increased above control for the full 2 h period investigated. The most marked increase was seen in arachidonic acid. The FFA in the hippocampus behaved in a similar way as in the cerebral cortex. Pretreatment with indomethacin did not influence the levels of FFA during seizures, nor did it influence the decrease in arachidonic acid seen after the initial increase. Thus, metabolism of arachidonic acid along the cyclooxygenase pathway does not seem to be responsible for the secondary decrease seen in arachidonic acid but not in the other FFAs. Possibly, unless selective reacylation of arachidonic acid is the only reason, peroxidation (Chan and Fishman 1980) and metabolism along the lipooxygenase pathway (Samuelsson 1980) may explain why arachidonic acid does not behave like the other FFAs. In the cerebellum, following an increase at twenty minutes of arachidonic acid as the only clear change, all values for the FFAs were close to the control values obtained at 2 h.

The regional changes in fatty acids thus behave similarly to changes in the cerebral energy state (I).

Paper III

The noradrenergic innervation in the cerebral cortex and hippocampus comes from the locus coeruleus. This innervation is inhibitory in the normal situation, partly regulating the total level of cAMP (Korf and Sebens 1979). We wanted to explore if lesions in the ascending NA system would modulate the cerebral metabolic response to bicuculline-induced status epilepticus.

The same metabolites as in paper I were here measured in animals with a lesion in the locus coeruleus system subjected to five minutes of seizures. In order to be able to dismiss the possibility that any differences seen between lesioned and sham-operated animals were due to a difference in seizure intensity we also closely monitored the EEG and measured CBF and CMRO₂ in a comparable series of animals. The assumption that phospholipase metabolism in the brain may be under the influence of the noradrenergic system (Bazán 1980, see also Hirata and Axelrod 1980) was also investigated by means of analysis of free fatty acids in lesioned and sham-operated animals.

As the EEG changes and the metabolic rate did not differ in the lesioned and sham-operated animals it was concluded that there was no difference in the seizure intensity between the two groups. This conclusion was corroborated by the fact that the levels of ATP were similar in the regions analyzed (cortex and hippocampus) both in lesioned and sham-operated animals. Analysis of FFA yielded no differences but a suggested a larger increase in the levels of arachidonic acid in the lesioned animals. This does not support the assumption that a major part of the phospholipase activity in the brain is mediated via the noradrenergic system. When comparing lesioned and sham-operated animals, the levels of cAMP were around 50 % both in the cortex and in the hippocampus and the corresponding levels for glycogen were 215 % and 170 % . The results suggest that a major part of the accumulation of cyclic AMP during status epilepticus is mediated by the noradrenergic locus coeruleus system. These results are also in line with the theory that phosphorylase activity is influenced by cAMP. The remaining capacity of the cells to break down glycogen after lesions in the locus coeruleus, is probably partly due to Ca²⁺ activity on phosphorylase b kinase.

Paper IV

This study was designed to answer two questions. First, do the results obtained with the 2-d-deoxyglucose method yield values for cerebral metabolic rate that are in agreement with values obtained with the Kety and Schmidt technique at normal and low metabolic rate? Secondly, what influence does 70 % N_2O have on the cerebral metabolic rate for glucose? The question is pertinent to the problem of the metabolic rate during seizures for the following reason. At the time when this study was done results from Sokoloff and coworkers suggested that metabolic rate in awake animals were higher than that predicted by $CMRO_2$ measurements from our own laboratory (Carlsson et al 1976). The results reported from that group also indicated that 70% N_2O considerably reduces metabolic rate. It therefore became of interest to measure CMR_{gl} under conditions identical to those where $CMRO_2$ data already was available.

The quantitative validity of the deoxyglucose technique at least during the control situation (paralyzed animals breathing N_2O/O_2) seems established as the results agree very well with the results obtained with two other techniques (Borgström et al 1976 and Berntman et al 1979 a). During phenobarbital anesthesia the glucose utilization rates obtained were somewhat less than expected as previous values for $CMRO_2$ spoke for a smaller suppression in metabolic rate. However this can be satisfactorily explained by the fact that barbiturate anesthesia inhibits the glycolysis at the phosphofructokinase step (Chapman et al 1978). Hence, part of the substrates for supporting oxygen consumption may be derived from endogenous stores of carbohydrate metabolites.

When comparing paralyzed, adrenalectomized animals breathing either N_2O or N_2 mixed with O_2 there was little difference in CMR_{gl} in the fronto-parietal cortical region, thus the results were in agreement with those obtained previously (Carlsson et al 1976). We conclude that N_2O has only small effects on the cerebral metabolic rate. It should be noted, though, that the N_2O increased the metabolic rate in some subcortical structures (see below).

Paper V

This paper deals with the effects of nitrous oxide *per se* on the local cerebral glucose utilization. The experiments performed gave information on two issues: What is the local cerebral glucose util-

ization in awake minimally restrained rats, and what is the influence of nitrous oxide per se on the metabolic rate. Knowledge of basal metabolic rates in the present rat strain is necessary for an evaluation of the relative increase in the metabolic rate encountered during seizures. It was of interest to know how the control situation used for the subsequent papers correlate with the metabolic rate in the awake state. Two groups of spontaneously breathing rats were studied, one breathing air and one N_2O/O_2 , using the model of Dahlgren et al (1981b)

The main finding in this paper was that the glucose metabolism was altered very little by replacing air with a N_2O/O_2 (70/30) gas mixture. The absolute values obtained in e.g. cortical areas are similar to those obtained in paralyzed and ventilated rats in the next paper. We therefore conclude that the comparison in metabolic rates between seizure rats and the paralyzed and ventilated control animals in the next paper would yield the same conclusions if the metabolic rate in seizure rats was compared to that in awake rats.

Paper VI

Local blood flow measurements were carried out in two groups of rats both subjected to 20 min of bicuculline induced seizures. One of the groups was injected with indomethacin. The purpose of the study was to quantify the differences in l-CBF after the induction of seizures and to see whether indomethacin influenced the circulatory response to seizures. Any effect caused by indomethacin (a commonly used cyclooxygenase blocker) was of interest for the following reasons: In both monkeys (Pickard and McKenzie 1973) and rats (Sakabe and Siesjö 1979) this drug had been shown to reduce resting CBF values and markedly attenuate the CBF response to hypercapnia, whereas it did not influence the CBF response to hypoxia. Was the circulatory response to seizures of the hypercapnic or the hypoxic type? Furthermore, since it was well established that both arachidonic acid and prostaglandins accumulate during seizures (II, Marion and Wolfe 1978) there was a possibility that arachidonic acid metabolites modulated the CBF response.

Since the changes in CBF after induction of 20 min seizures showed a marked interstructural variability we decided to postpone further measurements from longer seizure periods until the local metabolic rate could be measured as well (see below). Only the effects

of indomethacin will be dealt with here. In most parts of the brain there were no modifications seen in the indomethacin-treated animals. Thus, in that sense the CBF response resembled that seen in hypoxia rather than during hypercapnia. However it is striking that nucleus ruber, cerebellum and superior colliculus that had much lower values in the indomethacin-injected group all belong to the structures denoted as "hypometabolic" in paper VII. Thus, it seems that indomethacin may decrease the enhanced blood flow in structures which show little or no increase in metabolic rate, and which therefore show a disproportionate increase in CBF. Interestingly enough, the results suggest that the effect of indomethacin may be the opposite one in structures showing a marked increase in metabolic rate. The results on the metabolic rate/blood flow couple (see paper VII) justify more extensive study on the effects of indomethacin during seizures. However, since it now seems that indomethacin reduces blood flow by mechanisms that are not due to cyclooxygenase inhibition (Hougaard, Nilsson, Wieloch in preparation) such studies should be performed with other cyclooxygenase inhibitors as well and include measurements of both CBF and CMR_{gl} .

Paper VII

This paper describes in some detail changes in local cerebral glucose consumption and local blood flow during prolonged bicuculline-induced seizures. As described above, information on such changes is mandatory for an understanding of the coupling of the metabolic rate and blood flow during seizures, and may provide clues to the phenomenon of selective vulnerability.

Since the results on local metabolic rate are influenced by changes in the ratio of the distribution volumes between glucose and deoxyglucose, a short discussion on the validity of the technique and the procedure to overcome this problem is warranted. When the metabolic rate is markedly increased there is a decrease in the ratio of the tissue to plasma glucose concentrations. This will affect the lumped constant in equation 4. According to Pardridge et al (1982) the changes will be less at high plasma glucose values. Bicuculline seizures are fortunately accompanied by increases in the plasma levels of glucose. If the seizure activity is prolonged this increase is attenuated, and the plasma glucose concentration returns to control levels after 2 h. As this level is not sufficient to keep the changes

in the lumped constant at a reasonable magnitude, we included a second group of rats subjected to 2 h of bicuculline seizures. In this group the plasma glucose level was kept high by means of an intravenous infusion of glucose during the last hour of the experiment.

The CBF results described in this paper which represent extensions of those obtained in paper VI can be described as follows. At 20 min most structures had increased their flow rates 2.5- to 4-fold but the pattern was very heterogenous. The highest absolute values were observed in cerebral cortex, thalamus and in structures in the brain stem. At 60 min the pattern was similar. However, at 2 h a marked attenuation of the cerebral blood flow increase was noted. Several structures had in spite of ongoing seizure activity flow rates that were not different from control. This secondary reduction was most pronounced in the cerebral cortical (and limbic) structures. The results on I-CBF largely agree with the data reported by Horton et al (1980), but give by virtue of the autoradiographic technique a superior resolution at the local tissue level. Horton et al (1980) concluded that since the CBF was increased over control levels even after 2 h of seizures the epileptic brain damage can hardly be due to circulatory insufficiency but must be related to the maintained intense seizure discharge in itself. However as the present results demonstrate (see below) a mismatch between metabolic rate and blood flow develop in many structures when the seizures are prolonged.

After recalculation of the material on metabolic rate according to Pardridge et al (1982), it was possible to examine the coupling of CBF and CMR_{gl} at the local level. The structures examined could be divided into different groups. One group of structures which includes cerebral cortical regions and limbic structures was denoted "hypermetabolic", whereas most other regions measured belonged to a group denoted "hypometabolic". The main difference between the two groups was that in the first one the metabolic rate increased to values over 200% at two of the three times measured. The blood flow increase seemed to be in parity with the increase in metabolic rate at the beginning of the seizure period whereas, later, these structures developed a "relative hypoperfusion". The second group had smaller increases in the metabolic rate in spite of very large increases in blood flow. This hyperemic pattern persisted for the full 2 h. We note that the selectively vulnerable structures all fall in the first

group. However, one cannot draw the conclusion that the "relative hypoperfusion" is the cause of the cell damage seen after prolonged status epilepticus. Thus, cellular alterations are seen already after one hour, and at this time a "relative hyperperfusion" is still present in most parts of the brain.

Conclusions

1. In spite of the generalized nature of the seizures induced by the GABA receptor blocker bicuculline, metabolic perturbation was regionally different, being more severe in the cerebral cortex and hippocampus than in the cerebellum. The differences included both labile organic phosphates, glycolytic metabolites and free fatty acids.

2. In all probability such differences in metabolic perturbation reflected corresponding differences in local metabolic rate. However, the resolution of the autoradiographic technique used for measurement of local metabolic rate made it possible to distinguish a whole series of hyper- and hypo-metabolic structures with metabolic rates that varied between 1 and 4 times of the control values.

3. Changes in local cerebral blood flow during seizures were equally heterogenous. Unexpectedly, marked increases in CBF were encountered in structures with little or no increase in metabolic rate. In "hypermetabolic" structures the increase in blood flow were initially matched to that in metabolic rate, but later fell to yield a pattern of relative underperfusion.

4. Interruption of the noradrenergic innervation to the cerebral cortex or the hippocampus was found not to influence seizure activity or perturbation of the cerebral energy state. However the increase in cAMP normally seen during seizure activity, partly diminished, and as a result of this a reduction in glycogen breakdown was noted.

5. Pretreatment of animals with indomethacin had but small effects on the circulatory response to induced seizures. However the results suggested that the drug may reduce the blood flow in cerebral structures with a disproportionately large increase in CBF.

6. The results demonstrate that structures that are considered to show selective vulnerability during seizures (cerebral cortex and hippocampus) had a markedly increased metabolic rate, a clear perturbation of the energy metabolism and fatty acid metabolism and that a

relative hypoperfusion developed in prolonged seizures. In contrast a resistant structure (cerebellum) showed little evidence of metabolic perturbation, low metabolic rate, and a disproportionate increase in CBF. It remains to be shown whether a causal relationship exists.

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Kort sammanfattning

Ett intensivt studium ägnas sedan många år förändringar i hjärnans ämnesomsättning och circulation vid experimentellt framkallade epileptiska krampanfall. Bl.a. erbjuder krampanfall genom sina dramatiska effekter på ämnesomsättning och blodflöde lämpliga modeller för studier av kopplingen mellan neuronal aktivitet, ämnesomsättning och cirkulation. Vidare ger försök med olika krampframkallande medel värdefulla upplysningar om de cellulära mekanismer som leder till epileptisk aktivitet och epilepsiens spridning inom hjärnvävnaden. Slutligen erbjuder experimentella epilepsistudier outhärliga modeller för en analys av de faktorer som bidrar till att ge upphov till cellskador vid upprepade eller långdragna krämp.

I denna avhandling har försök gjorts att korrelera förändringar i ämnesomsättning och blodflöde regionalt i hjärnan med de tidigare kartlagda histopatologiska skador man ser efter ett utdraget kramp-anfall. Studierna har dels inkluderat analys av ämnesomsättningsprodukter i hjärnbarken, hippocampus och lilla hjärnan, dels utvärderat förändringar i lokal ämnesomsättning och blodflöde med kvantitativa autoradiografiska metoder efter långdragna krampanfall (status epilepticus). Bicuculline som är en GABA-blockerare har använts för att inducera krämp kemiskt. Eftersom metoden för att kartlägga lokal glucosomsättning i hjärnan är relativt ny inriktar sig två av arbetena på en validering av tekniken.

De regionala metabola förändringarna visar att det är de regioner som ökar sin ämnesomsättning mest under ett långdraget krampanfall som skadas av detsamma. Blodflödet ökar i hjärnan även i de delar vars ämnesomsättning icke avsevärt ökas. Ökningen i blodflöde är så stor att den i alla fall till en början väl motsvarar ökningen i ämnesomsättningshastighet. Efter två timmar ser man i de regioner som har haft högst ämnesomsättning en minskning av blodflödes-svaret vilket gör att man kan tala om en relativ underperfusion av vissa strukturer. Dessa strukturer råkar är de som drabbas av histopatologiska förändringar. Huruvida dessa fynd är den primära orsaken till cellskador eller ett sekundärt fenomen till andra patologiska processer får framtida studier utvisa.

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Metabolic Changes in Cerebral Cortex, Hippocampus, and Cerebellum During Sustained Bicuculline-Induced Seizures

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Abstract: The objective of the present experiments was to study metabolic correlates to the localization of neuronal lesions during sustained seizures. To that end, status epilepticus was induced by i.v. administration of bicuculline in immobilized and artificially ventilated rats, since this model is known to cause neuronal cell damage in cerebral cortex and hippocampus but not in the cerebellum. After 20 or 120 min of continuous seizure activity, brain tissue was frozen *in situ* through the skull bone, and samples of cerebral cortex, hippocampus, and cerebellum were collected for analysis of glycolytic metabolites, phosphocreatine (PCr), ATP, ADP, AMP, and cyclic nucleotides. After 20 min of seizure activity, the two "vulnerable" structures (cerebral cortex and hippocampus) and the "resistant" one (cerebellum) showed similar changes in cerebral metabolic state, characterized by decreased tissue concentrations of PCr, ATP, and glycogen, and increased lactate concentrations and lactate/pyruvate ratios. In all structures, though, the adenylate energy charge remained close to control. At the end of a 2-h period of status epilepticus, a clear deterioration of the energy state was observed in the cerebral cortex and the hippocampus, but not in the cerebellum. The reduction in adenylate energy charge in the cortex and hippocampus was associated with a seemingly paradoxical decrease in tissue lactate levels and with failure of glycogen resynthesis (cerebral cortex). Experiments with infusion of glucose during the second hour of a 2-h period of status epilepticus verified that the deterioration of tissue energy state was partly due to reduced substrate supply; however, even in animals with adequate tissue glucose concentrations, the energy charge of the two structures was significantly lowered. The cyclic nucleotides (cAMP and cGMP) behaved differently. Thus, whereas cAMP concentrations were either close to control (hippocampus and cerebellum) or moderately increased (cerebral cortex), the cGMP concentrations remained markedly elevated throughout the seizure period, the largest change being observed in the cerebellum. It is concluded that although the localization of neuronal damage and perturbation of cerebral energy state seem to correlate, the results cannot be taken as evidence that cellular energy failure is the cause of the damage. Thus, it appears equally probable that the pathologically enhanced neuronal activity (and metabolic rate) underlies both the cell damage and the perturbed metabolic state. The observed changes in cyclic nucleotides do not appear to bear a causal relationship to the mechanisms of damage. **Key Words:** Neuronal lesions—Status epilepticus—Bicuculline—Metabolic effects—Cerebral cortex—Hippocampus—Cerebellum. Folbergrová J. et al. Metabolic changes in cerebral cortex, hippocampus, and cerebellum during sustained bicuculline-induced seizures. *J. Neurochem.* 37, 1228–1238 (1981).

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Abbreviations used: CMRO₂, Cerebral oxygen utilization; EEG, Electroencephalogram; PCr, Phosphocreatine.

Recurrent or sustained seizures in man have been shown to cause neuronal cell damage, mainly localized to the neocortex, hippocampus, parts of thalamus and striatum, and to the cerebellum (Scholz, 1959; Norman, 1964; Corsellis and Meldrum, 1976). Due to the fact that neurons in these regions, particularly pyramidal cells in the neocortex and hippocampus and Purkinje cells in the cerebellum, are also damaged during other types of stress (e.g., hypoxia-ischemia), the term "selective vulnerability" has been used to denote their reaction to various insults. For the sake of convenience, we will refer to structures showing selective neuronal vulnerability as being "vulnerable" and to those not showing cell damage as "resistant."

Since it has remained a matter of speculation whether complicating factors such as hypoxia, hypotension, or hyperthermia contribute to the epileptic lesions seen in patients, several groups have studied histopathological alterations following drug-induced seizures in artificially ventilated animals in which systemic variables could be controlled. Although some authors have failed to observe neuronal cell damage after prolonged seizures (Purpura and Gonzales-Monteaquedo, 1960; Schwartz et al., 1970), such damage has been observed during seizures induced by bicuculline in baboons (Meldrum et al., 1973) and rats (Blennow et al., 1978). In the latter species, unequivocal neuronal damage was seen after 2 h of status epilepticus (for information on proportion of neurons damaged, see Söderfeldt et al., 1981). In both species, cell damage was observed in the neocortex and the hippocampus, and to some extent also in the thalamus and striatum, but was absent in the cerebellum. Thus, in contrast to, for example, hypoxia-ischemia, sustained seizures leave the vulnerable Purkinje cells intact, at least in well-oxygenated and normothermic animals.

The mechanisms causing epileptic cell damage are unknown. In the rat, bicuculline-induced status epilepticus has been found to be associated with a decrease in the cerebral cortical phosphocreatine and an increase in the lactate concentrations (with an associated rise in the lactate/pyruvate ratio); however, following an initial perturbation of the phosphorylation state of the adenine nucleotide pool, the adenylate energy charge remained close to control over a 2-h period of continuous seizure activity (Chapman et al., 1977). Furthermore, curtailment of cerebral oxygen supply (by induced hypoxia or hypotension) was found not to exaggerate the extent of cell damage (Blennow et al., 1978). On the basis of these results, depletion of cellular energy stores does not seem to be a likely cause of neuronal cell damage. However, the possibility remains that the moderate metabolic pertur-

bation observed in the cerebral cortex reflects overt energy failure in part of the neuronal population. Furthermore, it is conceivable that the failure of added hypoxia or hypotension to exaggerate tissue damage was due to a concomitant reduction in functional activity (i.e., neuronal metabolic rate). Finally, although the cerebral blood flow has been shown to be well above control values even in prolonged seizures (Horton et al., 1980), we have recently observed that selectively vulnerable areas such as the cerebral cortex and the hippocampus, with their greatly augmented metabolic rate, become relatively underperfused (Ingvar and Siesjö, in preparation).

In order to gain more insight into the biochemical mechanisms underlying epileptic cell damage in the brain, we induced seizures by bicuculline in immobilized and artificially ventilated rats, a model that has been found to cause neuronal cell damage after 1 and 2 h of seizure activity (Blennow et al., 1978; Söderfeldt et al., 1981) and then analyzed two "vulnerable" regions (the cerebral cortex and the hippocampus) and a "resistant" one (the cerebellum). The primary objective was to explore if a relationship exists between localization of cell damage and perturbation of tissue energy state, as reflected in the concentrations of glycolytic metabolites (glycogen, glucose, lactate, and pyruvate) and labile organic phosphates (phosphocreatine (PCr), ATP, ADP, and AMP). We found it of interest to measure concentrations of cyclic AMP (cAMP) and cyclic GMP (cGMP) as well. Thus, there is evidence suggesting that the concentration of cGMP bears some relationship to cell damage. Furthermore, alterations of levels of cyclic nucleotides might yield information on the balance between excitation and inhibition (see Discussion).

As the results will show, the resistant structure (cerebellum) maintained a more optimal energy state than the cerebral cortex and the hippocampal formation. However, the results disclosed that the latter structures developed signs of energy failure that were partly due to an insufficient substrate supply. To evaluate further the importance of this factor, an additional group was studied in which the animals were infused with glucose during the last hour of a 2-h period of status epilepticus. A preliminary account of some of the present findings has been given at a symposium devoted to status epilepticus (Siesjö et al., 1981).

MATERIALS AND METHODS

Animals

The experiments were performed on male Wistar rats of a SPF strain (Møllegaard Avlslaboratorium, Copenhagen) weighing 300–365 g. The animals had

free access to pellet food (Astra-Ewos, Södertälje) and tap water until the morning of the day of experiment.

Chemicals

Bicuculline methachloride as well as substrates and coenzymes for enzymatic, fluorometric analyses were obtained from Sigma Chemical Co. (St. Louis), lactate dehydrogenase from Millipore Co. (New Jersey), and all other enzymes from Boehringer and Sohn (Darmstadt). cAMP assays kits were from the Radiochemical Centre (Amersham) and cGMP (^{125}I -labeled) radioimmunoassay kits from New England Nuclear (Boston). All other chemicals were analytical grade.

Operative and Sampling Techniques

The animals were anesthetized in a jar with a gas mixture containing 3% halothane and 65% N_2O in oxygen. When unresponsive, they were tracheotomized, connected to a Starling-type respirator (Braun, Melsungen), paralyzed with tubocurarine chloride ($0.5 \text{ mg} \cdot \text{kg}^{-1}$, i.v.), and ventilated on 1% halothane and 70% N_2O in oxygen. Body temperature, as measured in the rectum, was kept close to 37°C by means of a heating bulb.

One femoral artery was cannulated for blood pressure recording and for sampling of blood for analyses of pH and blood gases, as well as of glucose concentration. One femoral vein was cannulated for injection of bicuculline and, whenever needed, for infusion of blood (see below). Holes were drilled in the skull bone and gold-plated screws were fastened for recording of the electroencephalogram (EEG) by bipolar leads on an Elema (Stockholm) EEG machine. A skin incision was placed over the cranial vault to accommodate a plastic funnel for later freezing of the brain *in situ* through the intact skull bone. It has previously been shown that, with this freezing technique, an optimal cerebral energy state is obtained also in deeply situated brain structures (Pontén et al., 1973). In order to avoid cooling of the brain, the incision was closed with clamps until just before freezing.

After the completion of the operative procedures, the halothane supply was discontinued and the animals were ventilated on 70% N_2O and 30% O_2 for at least 30 min before status epilepticus was induced. During this steady-state period, the body temperature was kept close to 37°C and arterial Po_2 and Pco_2 were kept above 90 mm Hg and in the range 35–40 mm Hg, respectively.

Induction and Maintenance of Seizures

At the end of the 30–40-min steady-state period, 3–5 ml of blood was slowly withdrawn to reduce mean arterial blood pressure from 140–150 to 95–110 mm Hg (this was done to reduce the ictal increase in pressure at the onset of seizures; see Chapman et al., 1977). Bicuculline was then injected i.v. in a dose of $1.2 \text{ mg} \cdot \text{kg}^{-1}$. Before injection, the bicuculline was dissolved in 0.1 M-HCl. The solution was then brought to a pH of about 5 and diluted with Krebs-Henseleit

solution to yield a concentration of $1.2 \text{ mg} \cdot \text{ml}^{-1}$. In all experiments, the initial pressure rise was followed by a slow decrease. In order to maintain mean arterial pressure at 110–120 mm Hg, the blood withdrawn plus donor blood was reinfused at the later stages of the seizure period as needed.

Methods and Analytical Techniques

Arterial Po_2 , Pco_2 , and pH were measured immediately after blood sampling with microelectrodes (Eschweiler and Co., Kiel, and Radiometer, Copenhagen) operated at 37°C , with due correction for any deviation in body temperature.

Tissue metabolites were determined in cerebral cortex, hippocampus, and cerebellum after seizure periods of 20 and 120 min. For dissection of tissue samples, the brains (stored in liquid nitrogen) were allowed to reach a temperature of -22°C . Cerebellar cortex (with underlying white matter) was separated from the vermis, and a 20–25-mg sample was collected. A sample of similar size was obtained from the cerebral cortex (parietal-sensorimotor), care being taken to exclude the cingulate cortex. The hippocampal sample was collected from a coronal strip of about 3 mm taken 2 mm frontal to the occipital pole. All tissue not belonging to the hippocampal formation was then trimmed off and a 20–25-mg sample was taken for extraction.

Following weighing on a torsion balance, each tissue sample was extracted with HCl-methanol at -22°C and subsequently with perchloric acid at 0°C . After centrifugation and neutralization of the extracts with a KOH-imidazole base-KCl mixture, enzymatic, fluorometric techniques were used to determine tissue contents of glycogen, glucose, lactate, pyruvate, PCr, ATP, ADP, and AMP (for details of procedure, see Folbergrová et al., 1972*a,b*). Similar techniques were used to measure glucose in arterial blood.

A similar extraction technique was used to measure cAMP with a protein-binding technique. For the measurements of cGMP an acidic extract was prepared (Lowry and Passonneau, 1972). Potassium bicarbonate and acetate buffer were added (pH 6.2). cGMP was then measured with radioimmunoassay as described by Steiner et al. (1972).

Calculations

The energy charge of the adenine nucleotide pool was calculated according to Atkinson (1968). Statistical differences were evaluated with Student's *t*-test.

RESULTS

Two series of animals were studied, each with its own control group. In the first, brains were frozen for analysis after 20 and 120 min of status epilepticus, respectively. In these, blood glucose concentrations were not measured. Because very low tissue glucose concentrations were observed in some animals, a second series of animals was infused with glucose (0.5 ml of

50% glucose solution/h) during the second hour of status epilepticus.

Physiological Variables

Physiological variables in the two series, as measured prior to freezing of tissue, are shown in Table 1. In both series, body temperature and arterial Po_2 were similar. Infusion of previously withdrawn or donor blood maintained blood pressure of seizure animals at 110–120 mm Hg. Arterial Pco_2 fell after 20 min of seizures but was close to control after 2 h. Arterial pH was reduced during seizures. In the second, glucose-infused series, blood glucose concentrations of control animals ranged between 9.0 and 14.4 $\mu\text{mol}\cdot\text{g}^{-1}$. In seizure animals the corresponding variation was between 5.9 and 16.2 $\mu\text{mol}\cdot\text{g}^{-1}$. In the first series, peak (ictal) mean arterial blood pressures varied between 145 and 190 mm Hg; in the second series, the corresponding range was 160–195 mm Hg.

In all seizure animals, the EEG changes were similar to those previously recorded (Chapman et al., 1977). Thus, following the intense, initial seizure discharge, an EEG pattern of bursts and suppression continued unabated throughout the seizure periods studied.

Regional Metabolite Concentrations

Cerebral Cortex and Hippocampus

Table 2 gives the concentrations of labile organic phosphates, glycolytic metabolites, and cyclic nucleotides in the first series of animals.

With one exception, control values for the cerebral cortex were those usually obtained in this laboratory (e.g., Chapman et al., 1977). In both series (see Table 2 and below), the lactate concentrations were about 1 $\mu\text{mol}\cdot\text{g}^{-1}$ higher, with a corresponding elevation of the lactate/pyruvate ratios. This was due to the fact that a few animals had unusually high lactate concentrations, the highest being 4.1 $\mu\text{mol}\cdot\text{g}^{-1}$. In all probability, these high values were artefactual. In support, a third series of control animals ($n = 6$) in which a larger cortical sample was taken and immediately processed for analysis had lactate concentrations of $1.73 \pm 0.10 \mu\text{mol}\cdot\text{g}^{-1}$. We conclude, therefore, that the true control values for lactate were below 2 $\mu\text{mol}\cdot\text{g}^{-1}$. Since animals with high lactate levels had normal values for PCr, ATP, ADP, and AMP, and since the variability in control lactate levels had no influence on the conclusions drawn, we made no attempts to eliminate the error.

After 20 min of seizure activity, changes in the cerebral cortex concentrations of labile phosphates, glycogen, glucose, lactate, and pyruvate were those expected from previous results (Chapman et al., 1977). Thus, significant alterations were observed in the PCr, glycogen, lactate, and pyruvate concentrations, as well as in the lactate/pyruvate ratio. Changes in adenine nucleotide concentrations were confined to a moderate decrease in ATP concentration, while the reduction in adenylate energy charge did not reach statistical significance. In confirmation of many previous studies of electrical or drug-induced seizures (see Discussion), we found increased concentrations of cyclic nucleotides.

TABLE 1. Body temperature, mean arterial blood pressure (MABP), arterial blood gases, pH, and glucose concentrations in control and experimental groups

Group	Temp. (°C)	MABP (mm Hg)	Pao_2 (mm Hg)	Paco_2 (mm Hg)	pH	Glucose ($\mu\text{mol}\cdot\text{g}^{-1}$)
First Series						
Control	36.8 ± 0.2	164 ± 3	108 ± 1	36.5 ± 0.6	7.39 ± 0.02	—
20 min	36.4 ^a ± 0.1	120 ^c ± 1	116 ± 2	28.0 ^b ± 1.8	7.08 ^c ± 0.02	—
120 min	37.1 ± 0.2	115 ^c ± 3	106 ± 2	32.7 ^a ± 1.2	7.11 ^c ± 0.04	—
Second Series						
Control	37.2 ± 0.1	156 ± 3	114 ± 2	37.6 ± 0.9	7.34 ± 0.03	12.1 ± 0.9
120 min	37.2 ± 0.1	112 ^c ± 3	117 ± 7	35.2 ± 1.0	7.11 ^b ± 0.04	10.9 ± 1.6

The values are means $\pm$ S.E.M. ($n = 6$ in each group). In the second series, a slow infusion of glucose was given during the last one-h period (see Materials and Methods).

Statistical significance with respect to controls: ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$.

TABLE 2. *Labile metabolites in the cerebral cortex and hippocampus after 20 and 120 min of bicuculline-induced status epilepticus in animals that did not receive glucose during seizures*

Region	Metabolite	Group		
		Control	20-min	120-min
Cerebral cortex	Phosphocreatine	4.95 ± 0.09	3.50 ± 0.19 ^a	2.85 ± 0.20 ^a
	ATP	2.80 ± 0.05	2.60 ± 0.05 ^a	2.50 ± 0.08 ^b
	ADP	0.303 ± 0.006	0.305 ± 0.011	0.448 ± 0.028 ^a
	AMP	0.043 ± 0.002	0.045 ± 0.004	0.093 ± 0.020 ^a
	Energy charge	0.938 ± 0.002	0.932 ± 0.003	0.895 ± 0.012 ^a
	Glycogen	2.68 ± 0.26	0.48 ± 0.08 ^a	0.78 ± 0.23 ^a
	Glucose	3.43 ± 0.27	2.07 ± 0.22 ^b	0.92 ± 0.16 ^a
	Lactate	2.58 ± 0.37	10.28 ± 0.46 ^a	7.35 ± 1.71 ^a
	Pyruvate	0.076 ± 0.003	0.136 ± 0.003 ^a	0.119 ± 0.015
	Lactate/pyruvate	34.2 ± 5.1	75.9 ± 3.7 ^a	58.3 ± 8.2 ^a
	cAMP	1.80 ± 0.06	2.24 ± 0.04 ^a	2.25 ± 0.18 ^a
	cGMP	0.101 ± 0.012	0.280 ± 0.050 ^b	0.327 ± 0.050 ^a
Hippocampus	Phosphocreatine	5.25 ± 0.10	3.99 ± 0.25 ^a	4.04 ± 0.24
	ATP	2.93 ± 0.02	2.65 ± 0.08 ^a	2.61 ± 0.05 ^a
	ADP	0.300 ± 0.018	0.354 ± 0.038	0.434 ± 0.069
	AMP	0.044 ± 0.006	0.056 ± 0.005	0.073 ± 0.012
	Energy charge	0.941 ± 0.002	0.925 ± 0.005 ^a	0.909 ± 0.012 ^a
	Glycogen	3.30 ± 0.32	0.48 ± 0.08 ^a	1.78 ± 0.48 ^a
	Glucose	3.14 ± 0.29	1.60 ± 0.24 ^b	0.84 ± 0.20 ^a
	Lactate	1.85 ± 0.06	8.96 ± 0.54 ^a	4.63 ± 0.70 ^b
	Pyruvate	0.077 ± 0.007	0.132 ± 0.006 ^a	0.104 ± 0.015
	Lactate/pyruvate	23.5 ± 1.6	68.2 ± 3.1 ^a	45.6 ± 4.9 ^a
	cAMP	1.17 ± 0.07	1.37 ± 0.07	1.24 ± 0.14
	cGMP	0.104 ± 0.019	0.398 ± 0.144	0.374 ± 0.123

The values are means ± S.E.M. Cyclic nucleotide concentrations are in $\mu\text{mol} \cdot \text{kg}^{-1}$, all other metabolite concentrations in $\mu\text{mol} \cdot \text{g}^{-1}$ of wet weight.

^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$.

In contrast to our previous findings (Chapman et al., 1977), status epilepticus of 2-h duration was found to be associated with a further deterioration of cerebral cortical energy state, and glycogen concentration failed to normalize. Unexpectedly, the reduction in adenylate energy charge did not lead to further accumulation of lactic acid (see below). At that time, a threefold increase in cGMP concentration persisted and a moderate rise in cAMP concentration seemed to occur (see below).

In the hippocampus, metabolic changes after 20 min of seizures were similar to those observed in the cerebral cortex. The concentrations of PCr and ATP were reduced, and although the changes in ADP and AMP did not reach statistical significance, that in the energy charge did. Lactate and pyruvate concentrations rose, as did the lactate/pyruvate ratio, and glucose and glycogen concentrations fell, the latter to 15% of control. In contrast to the cerebral cortex, the hippocampus showed no significant increase in cAMP concentration. There was an unusually large variability in cGMP concentrations. As a result of this, the three- to fourfold increases in the mean value after 20 and 120 min did not reach statistical significance (Student's *t*-test). With

few exceptions, changes in the hippocampus after 2 h of status epilepticus were similar to those observed in the cerebral cortex. Features in common were not only the perturbation of cerebral energy state with reductions in the PCr and ATP concentrations, as well as in the adenylate energy charge, but also the pronounced fall in tissue glucose concentrations. Furthermore, the data suggest a persisting threefold to fourfold rise in cGMP concentration. One difference was that the hippocampus showed a more extensive resynthesis of glycogen; another was that the cAMP concentration did not differ from control.

In some animals glucose concentrations in the cerebral cortex and the hippocampus were below $1 \mu\text{mol} \cdot \text{g}^{-1}$, suggesting that the glucose supply had become limiting for cellular energy production, lactate formation, and glycogen synthesis. This suggestion was borne out when these variables were correlated to tissue glucose concentrations in the 2-h seizure groups (Fig. 1). Thus, in animals in which these concentrations fell well below $1 \mu\text{mol} \cdot \text{g}^{-1}$, a clear reduction in energy charge was observed, and this was correlated to a paradoxical reduction in lactate concentrations. Furthermore, in both cortex and hippocampus, the glycogen concentrations cor-

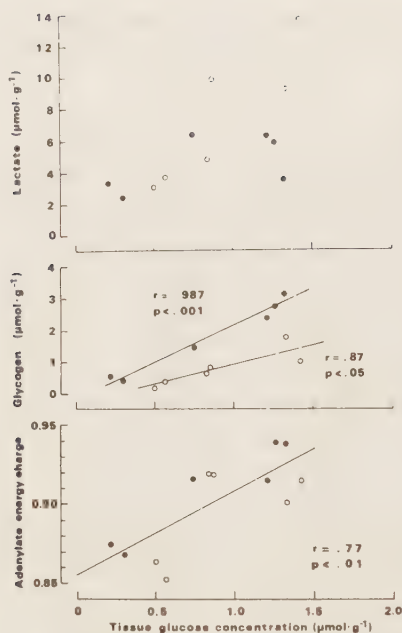


FIG. 1. Individual values for tissue levels of lactate, glycogen, and adenylate energy charge against the tissue level of glucose in animals subjected to 2 h of status epilepticus not receiving glucose infusion. Unfilled symbols denote cortex and filled symbols, hippocampus.

related to the glucose levels. A further point worth noting was that in those 3 animals that had the lowest cerebral cortical energy charge values (0.901, 0.864, and 0.853), the cAMP concentrations were clearly elevated (2.21, 2.72, and 2.84 $\mu\text{mol}\cdot\text{kg}^{-1}$, respectively). In the remaining 3 animals, the cAMP concentrations were within the control range, indicating that the increase in cAMP concentration correlated to the deterioration of cellular energy state.

The results obtained in individual animals showed that in those 2 animals having the highest tissue glucose concentrations, the hippocampal energy charge values fell within the control range. The question arose, therefore, whether limitation of glucose supply was the sole reason for the reduction in energy charge. Accordingly, we analyzed key metabolites in the cerebral cortex and the hippocampus in animals infused with glucose during the second hour of a 2-h status epilepticus period. The results are shown in Table 3. In these animals, changes in PCr and lactate concentrations (and in the lactate/pyruvate

ratios) were similar to those recorded in series 1 (see Table 2 above), but the reduction in energy charge was less pronounced, and ATP concentrations were not reduced. However, since ADP and AMP concentrations rose in both cerebral cortex and hippocampus, the energy charge was significantly reduced.

The results of Fig. 2, which relate tissue glucose concentrations to energy charge values and lactate concentrations, allow two conclusions. First, even if "adequate" tissue glucose concentrations are upheld, the energy charge of both cerebral cortex and hippocampus are reduced below the control range. Second, a clear further reduction in energy charge is not observed until tissue glucose concentrations fall below about 1 $\mu\text{mol}\cdot\text{g}^{-1}$. Obviously, at these concentrations glucose supply is also insufficient to maintain lactic acid production (cf. Fig. 1 above). However, a comparison of results obtained in the two series indicates that tissue glucose concentrations do not adequately reflect substrate delivery. Thus, although two of the glucose-infused animals had very low tissue glucose concentrations, the corresponding energy charge values were clearly higher than those obtained in the first series (cf. Figs. 1 and 2).

The Cerebellum

In the cerebellum, a seizure period of 20 min was associated with a highly significant decrease in the PCr and a small reduction in the ATP concentration (Table 4). The lactate concentration was clearly elevated, as was the lactate/pyruvate ratio, but the adenylate energy charge did not deviate from control. Further evidence of the involvement of cerebellar tissue in the general metabolic perturbation accompanying the seizure discharge was the extensive breakdown of glycogen. In contrast to the cerebral cortex and the hippocampus, though, the cerebellum showed a normal cerebral energy state, and extensive re-synthesis of glycogen, after 2 h of status epilepticus. It is of interest that glucose concentrations in the tissue were almost 4 times as high as in the cerebral cortex and the hippocampus.

The results suggest that the seizures did not affect the cAMP system of the cerebellum. Thus, in the 20-min group; only two values and in the 2-h group but a single value were outside the normal range. However, massive increases in cGMP content occurred, the concentrations at 20 and 120 min being 1400 and 450% of control, respectively.

DISCUSSION

Some previous data have been published bearing on regional metabolic changes induced by

TABLE 3. Labile metabolites in the cerebral cortex and the hippocampus after 120 min of bicuculline-induced status epilepticus in glucose-infused animals

Region	Metabolite	Group	
		Control	120-min
Cerebral cortex	Phosphocreatine	5.03 ± 0.14	3.32 ± 0.18 ^b
	ATP	2.86 ± 0.06	2.82 ± 0.03
	ADP	0.306 ± 0.002	0.355 ± 0.011 ^a
	AMP	0.041 ± 0.003	0.062 ± 0.004 ^a
	Energy charge	0.939 ± 0.001	0.926 ± 0.003 ^a
	Glucose	4.48 ± 0.46	1.26 ± 0.25 ^b
	Lactate	2.56 ± 0.43	9.04 ± 1.31 ^b
Hippocampus	Pyruvate	0.087 ± 0.008	0.130 ± 0.060 ^a
	Phosphocreatine	5.19 ± 0.13	3.97 ± 0.12 ^b
	ATP	2.81 ± 0.02	2.74 ± 0.04
	ADP	0.286 ± 0.009	0.342 ± 0.006 ^b
	AMP	0.049 ± 0.001	0.061 ± 0.002 ^b
	Energy charge	0.939 ± 0.001	0.927 ± 0.001 ^b
	Glucose	4.07 ± 0.37	1.20 ± 0.27 ^b
	Lactate	1.38 ± 0.16	6.82 ± 0.63 ^b
	Pyruvate	0.090 ± 0.005	0.116 ± 0.005 ^a

The values are means ± S.E.M. All metabolite concentrations in $\mu\text{mol} \cdot \text{g}^{-1}$ of wet weight.

^a $p < 0.01$, ^b $p < 0.001$.

electroshock (Ferrendelli and McDougal, 1971a) and audiogenic seizures in susceptible animals (Ferrendelli and McDougal, 1971b), as well as those induced by methionine sulfoximine (Folbergrová et al., 1969) and flurothyl (Duffy et al., 1975). Apart from the fact that audiogenic sei-

zures were found to cause more pronounced changes in subcortical than in cortical structures, these studies revealed that the cerebellum and brainstem gave changes similar (albeit somewhat less pronounced) than those observed in the cerebral cortex or the forebrain. No previous results seem to have been published on more sustained seizures, and we lack data on hippocampus for other metabolites than cyclic nucleotides (Ferrendelli and Kinscherf, 1977; Ferrendelli et al., 1980).

The present results demonstrate that after 20 min of seizure activity, all three structures analyzed showed metabolic changes similar to those previously recorded in the cerebral cortex (Chapman et al., 1977). These changes were dominated by decreases in the tissue concentrations of PCr and glycogen and by increases in the lactate concentrations and lactate/pyruvate ratios, whereas the adenylate energy charge remained close to control values. After 2 h of status epilepticus the situation was changed, since two structures (cerebral cortex and hippocampus) showed a further deterioration of cerebral energy state, whereas the third (cerebellum) had a normal metabolic state. We will discuss these results under three headings.

Energy State and Substrate Supply

In the present study, the deterioration of cerebral cortical energy state after 2 h of status epilepticus was more pronounced than in a previous one (Chapman et al., 1977; see also Blennow et al., 1977). The results obtained clearly indicate that a restricted substrate supply must

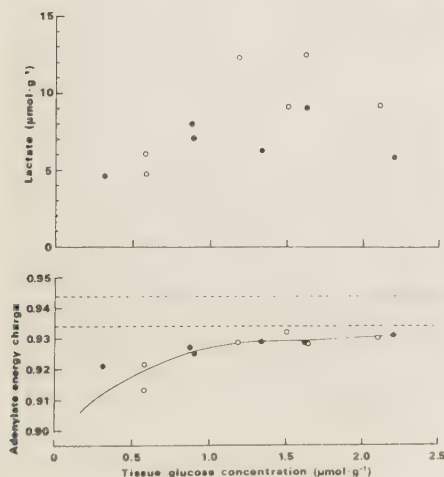


FIG. 2. Individual values for tissue levels of lactate and adenylate energy charge against the tissue level of glucose at 2 h of status epilepticus when glucose was infused during the last hour of the experiment. The line in the table for adenylate energy charge is drawn for visual best fit. Unfilled symbols denote cortex and filled symbols, hippocampus.

BRAIN METABOLISM IN SEIZURES

TABLE 4. *Labile metabolites in cerebellar cortex after 20 and 120 min of bicuculline-induced status epilepticus in animals that did not receive glucose during seizures*

Metabolite	Group		
	Control	20 min	120 min
Phosphocreatine	7.02 ± 0.16	5.98 ± 0.07 ^a	7.10 ± 0.20
ATP	2.61 ± 0.03	2.47 ± 0.03 ^b	2.61 ± 0.06
ADP	0.241 ± 0.010	0.249 ± 0.007	0.253 ± 0.003
AMP	0.039 ± 0.002	0.039 ± 0.002	0.039 ± 0.002
Energy charge	0.945 ± 0.002	0.941 ± 0.001	0.943 ± 0.002
Glycogen	3.82 ± 0.23	0.67 ± 0.11 ^c	2.86 ± 0.12 ^b
Glucose	4.37 ± 0.33	4.35 ± 0.53	2.45 ± 0.39 ^a
Lactate	1.49 ± 0.21	6.67 ± 0.69 ^a	2.31 ± 0.21 ^a
Pyruvate	0.070 ± 0.004	0.144 ± 0.011 ^c	0.088 ± 0.005 ^a
Lactate/pyruvate	21.0 ± 2.5	46.3 ± 2.4 ^c	26.2 ± 1.5
cAMP	0.99 ± 0.07	1.39 ± 0.21	1.03 ± 0.21
cGMP	0.590 ± 0.106	8.230 ± 1.059 ^a	2.676 ± 0.508 ^a

The values are means ± S.E.M. Cyclic nucleotide concentrations are in $\mu\text{mol} \cdot \text{kg}^{-1}$, all other metabolite concentrations in $\mu\text{mol} \cdot \text{g}^{-1}$ of wet weight.

^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$.

have contributed to this difference. Thus, the adenylate energy charge varied directly with the tissue glucose concentrations. Furthermore, in animals having low glucose concentrations, the lactate concentrations decreased and signs of glycogen resynthesis were lacking. The results qualitatively resemble those obtained in one previous study, in which seizures were induced in food-deprived animals (Blennow et al., 1979). In these, which developed hypoglycemia during the course of the seizures, the adenylate energy charge values were similar to those recorded presently, levels of lactate and other carbohydrate metabolites were low, and no glycogen resynthesis occurred. However, when blood glucose concentrations fell below about $3 \mu\text{mol} \cdot \text{g}^{-1}$, the EEG changed from a burst-suppression pattern to one with single spikes and cerebral oxygen utilization (CMRO_2) decreased to control values. This is in contrast to the present experiments, in which a burst-suppression pattern was maintained and CMRO_2 was upheld at values exceeding 200% of control (Ingvar and Siesjö, in preparation). Obviously, in some of the present experiments the curtailment of substrate supply was sufficient to reduce the phosphorylation state of the adenine nucleotide pool but too moderate to affect the seizure activity (or the metabolic rate). It is of interest that a further restriction of the glucose supply does not seem to exaggerate cellular energy failure (see Blennow et al., 1979). This indicates that attenuation of seizure discharge, with an associated reduction in metabolic rate, provides a mechanism that prevents more extensive energy failure from occurring.

It has previously been shown that tissue glucose concentrations are markedly reduced in immature animals during electroshock- or fluoro-

thyl-induced seizures and that glucose administration reduces mortality and ameliorates the retardation of brain development (Wasterlain and Duffy, 1976). No such beneficial effect was observed in mature animals. Since failure of cerebral glucose supply has not previously been observed in normally fed, mature animals during status epilepticus, the present results were unexpected. We therefore did not measure blood glucose concentrations in the first series. However, a parallel series of animals in which local glucose consumption was measured (Ingvar and Siesjö, in preparation) showed that some animals had blood glucose concentrations as low as $4\text{--}6 \mu\text{mol} \cdot \text{g}^{-1}$. The results obtained in the present series indicate that substrate supply becomes limiting for metabolism in intensely hypermetabolic structures when tissue glucose concentrations are reduced well below $1 \mu\text{mol} \cdot \text{g}^{-1}$. Data obtained in glucose-infused animals indicate that this occurs when blood glucose concentrations are reduced below about $10 \mu\text{mol} \cdot \text{g}^{-1}$. In other words, with the intense neuronal activity encountered in bicuculline-induced seizures, an adequate substrate supply requires a blood glucose concentration of about 200% of control. Inspection of data reported by Chapman et al. (1977) reveals that glucose concentrations were close to this critical level and indicates that subtle differences in nutritional state may explain the differences in results.

Local Energy Metabolism and Neuronal Cell Damage

The present results have demonstrated that after 2 h of continuous seizure activity, tissue energy state was perturbed in two regions (cerebral

cortex and hippocampus) but virtually normal in the third (cerebellum). Since neuronal cell damage is observed in the cortex and hippocampus but not in the cerebellum (see Introduction), it is tempting to conclude that cellular energy failure is a contributing factor. Additional results favoring such a conclusion are those demonstrating that the vulnerable regions (cerebral cortex and hippocampus) develop a relative underperfusion during prolonged seizures, with an indicated mismatch between metabolic rate and blood flow (Ingvar and Siesjö, in preparation).

The question arises whether the energy state of the cerebellum was better preserved than in the cortex or hippocampus because cerebellum received an adequate glucose supply. In all probability, this is not so. Thus, even in animals in which the glucose supply was sufficient to maintain cerebral cortical lactate levels at around $10 \mu\text{mol}\cdot\text{g}^{-1}$, the energy charge values were clearly subnormal. Furthermore, in contrast to the cerebellum, the cerebral cortex and the hippocampus had raised lactate concentrations and increased lactate/pyruvate ratios. Some seizure-induced metabolic changes seemed somewhat less pronounced in the hippocampus than in the cerebral cortex. Thus, even in animals with high glucose concentrations, lactate values were lower than in the cerebral cortex, a more extensive resynthesis of glycogen occurred, and cAMP was not increased above control (see below). However, since metabolic rate is more heterogeneous in the hippocampal formation than in the cerebral cortex (Ingvar and Siesjö, in preparation), these differences do not exclude the possibility of a relatively pronounced metabolic perturbation in part of the neuronal population of the hippocampus.

In spite of these considerations, it may be premature, if not unwise, to conclude that cellular energy failure underlies the neuronal lesions observed in the neocortex and the hippocampus. Thus, it appears equally probably that the mechanisms of damage are related to the excessively enhanced functional activity (and metabolic rate) and that energy failure, rather than being the cause of the cell damage, is just the result of cellular energy demands that outstrip the supply. By the same token, the relative deterioration of capillary perfusion could reflect cellular and vascular changes secondary to the metabolic imbalance. Clearly, the problem of cause and effect can only be settled by further experiments.

Changes in Cyclic Nucleotides

Many previous investigations have shown that seizures induced by a variety of means raise the tissue concentrations of cAMP and cGMP (Sat-

tin, 1971; Folbergrová, 1975, 1980; Lust et al., 1976; Ferrendelli and Kinscherf, 1977; Raabe et al., 1978; Gross and Ferrendelli, 1980; Ferrendelli et al., 1980). At present, it is not possible to offer a straightforward interpretation of these findings, but there is evidence suggesting that changes in cAMP and cGMP to some extent reflect the balance of inhibition and excitation in the tissue. There is evidence that the main physiological activators of adenylate cyclase in the brain are catecholamines, chiefly noradrenaline and adenosine (see Bloom, 1975; Iversen, 1977; Goldberg and Haddox, 1977). The coupling of noradrenaline and cAMP and the fact that both inhibit firing rates of many cerebral neurons have led to the view that an increase in cAMP concentration may be related to synaptic inhibition. This assumption is corroborated by findings showing that interference with cerebral noradrenergic systems decreasing turnover of noradrenaline in the brain reduces seizure thresholds (Mason and Corcoran, 1979; Corcoran and Mason, 1980). The cGMP system has been shown to be affected by a variety of neurotransmitters and neurohormones, as well as by nonhormonal agents such as oxidants, fatty acids, and cations (see Goldberg and Haddox, 1977; Murad et al., 1979; Ohga and Daly, 1977). There is evidence suggesting that the most important activator of guanylate cyclase is intracellular Ca^{2+} (Ferrendelli et al., 1976; see also Briggs and DeRubertis, 1980). In contrast to cAMP, cGMP has been assumed to mediate excitation, partly by its presumed association with cholinergic pathways (see Stone et al., 1975, but also Phillis, 1977).

We have previously observed that cerebral cortical concentrations of cAMP increase about sixfold after 1 min of seizure discharge, remain moderately elevated after 30 min, and normalize after 60 min (Siesjö et al., 1981). These results, and those observed in the present 20-min group, demonstrate that cAMP concentrations normalize during the first hour and increase again after 2 h of status epilepticus. Concerning the mechanism involved in the accumulation of cAMP, we tentatively conclude that it could reflect the perturbation of cerebral phosphorylation potential and the release of noradrenaline from storage sites (see Calderini et al., 1978), as well as dephosphorylation of AMP with release of adenosine, but the participation of other mediator(s) cannot be excluded at present.

The sustained elevation of cGMP concentration is consistent with results showing an influx of Ca^{2+} from extracellular fluids during seizures (Heinemann et al., 1977; see also Nicholson, 1979). Some results suggest that Ca^{2+} may increase guanylate cyclase activity indirectly, notably by triggering hydrolysis of phospholipids,

the primary activators of guanylate cyclase activity being hydroperoxide breakdown products of arachidonic acid (for reviews, see Goldberg and Haddox, 1977; Murad et al., 1979). If this is so, increased cGMP concentrations could mirror the cellular production of potentially toxic arachidonate metabolites (Borgeat and Samuelsson, 1979). This hypothesis is consistent with results showing that seizures are accompanied by release of free fatty acids, chiefly arachidonic acid (Bazán, 1970; Marion and Wolfe, 1978; Chapman et al., 1980).

At first sight, therefore, changes in cyclic nucleotide concentrations in cerebral cortex and hippocampus would seem to reflect a disturbed balance between excitation and inhibition during prolonged bicuculline-induced seizures, and possibly also the continued production of free radical and other potentially harmful breakdown products of polyenoic fatty acids. However, these conclusions become untenable in view of the fact that the cerebellum, a relatively nonepileptogenic structure showing little increase in metabolic rate and virtually no accumulation of free fatty acids (Siesjö et al., 1981), had the most pronounced changes in cGMP/cAMP ratio. Clearly, the interpretation of changes in cyclic nucleotide concentrations during seizures must await further results.

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The Influence of Bicuculline-Induced Seizures on Free Fatty Acid Concentrations in Cerebral Cortex, Hippocampus, and Cerebellum

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Abstract: Using ventilated rats maintained on N_2O-O_2 (70:30, vol/vol) we induced continuous seizures with i.v. bicuculline and analysed free fatty acids (FFA) in cerebral cortex, hippocampus, and cerebellum after seizure durations of 1–120 min. In the cerebral cortex, peak FFA concentrations were observed after 5 min, with a threefold increase in total FFA content. The values then remained unchanged for the next 15–20 min, but decreased thereafter. At 60 and 120 min, total FFA contents were only moderately increased above control. In the initial period, arachidonic acid increased about 10-fold and stearic acid 2- to 3-fold, with little change in palmitic acid and linoleic acid concentrations. At all times, the docosahexenoic acid concentration was markedly increased. Following its massive accumulation at 1 min, arachidonic acid gradually decreased in concentration. Pretreatment of animals with indomethacin did not alter this behaviour. After 20 and 120 min of seizure activity, changes in total and individual FFA concentrations in the hippocampus were similar to those observed in the cerebral cortex. The cerebellum behaved differently. Thus, at 20 min the only significant change was a 5- to 10-fold increase in arachidonic acid concentration and, after 120 min, total and individual FFA concentrations were similar to control values. Furthermore, since the control values for arachidonic acid were much lower in the cerebellum, the 20-min values were only about 20% of those observed in the cerebral cortex and the hippocampus. **Key Words:** Seizure activity—Free fatty acids—Bicuculline. Siesjö B. K. et al. The influence of bicuculline-induced seizures on free fatty acid concentrations in cerebral cortex, hippocampus, and cerebellum. *J. Neurochem.* 39, 796–802 (1982).

In 1968, Lunt and Rowe isolated and characterised a pool of free fatty acids (FFA) in the brain. Bazán and co-workers subsequently showed that the tissue FFA concentrations, which are normally low (about $150 \mu\text{mol} \cdot \text{g}^{-1}$), increase precipitously in ischemia and in seizures induced by electroshock or pentylenetetrazol, arachidonic acid (20:4) and stearic acid (18:0) showing the largest relative changes (Bazán, 1970; 1971; 1976; Bazán et al. 1971). The authors also produced evidence to show that the FFA released mainly emanated from phospholipids, probably by increased activity of phospholipases of the A_2 type (see Bazán, 1970). These original observations have subsequently been con-

firmed by several other groups (for documentation and further references, see Marion and Wolfe, 1978; 1979; Porcellati et al., 1978; Yoshida et al., 1980; Rehnrcrona et al., 1982), and it has been shown that a similar increase in tissue FFA content occurs in severe hypoglycemia as well (Agardh et al., 1981a,b). Recent results have shown that, at least in ischemia, a large fraction of the arachidonic acid released is derived from phosphatidylinositol and phosphatidylcholine (Marion and Wolfe, 1979), two phospholipids with a rapid turnover (Sun et al., 1979).

Since previous studies concerned with FFA metabolism in the brain during seizures were carried

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Abbreviation used: FFA, Free fatty acid.

out on spontaneously breathing animals without control of physiological variables (Bazán, 1970; 1971; Marion and Wolfe, 1978), the question arose whether the changes observed were due to the seizure discharge per se, or to complicating hypoxia or hypotension. However, by inducing seizures in ventilated and well-oxygenated animals (by i.v. administration of bicuculline) we could show that FFA, and particularly arachidonic acid, accumulated in spite of the fact that the phosphorylation state of the adenine nucleotide system was close to normal (Chapman et al., 1977; 1980; Folbergrová et al., 1981).

The present experiments, carried out on ventilated rats with continuous bicuculline-induced seizures, were designed to provide answers to the following questions. (1) What is the time course of changes in total and individual FFA concentrations following their accumulation at 1 min? (2) Can the secondary decrease in arachidonic acid concentration be influenced by indomethacin, a potent inhibitor of cyclo-oxygenase? (3) Is there a difference in FFA accumulation between, on one hand, the cerebral cortex and hippocampus (two structures showing neuronal cell damage) and, on the other hand, the cerebellum (a structure without signs of cell damage)? A preliminary account of some of the findings has been given (Siesjö et al., 1982).

MATERIALS AND METHODS

Experimental groups

Two series of animals were studied. *Series A* consisted of one control group and three experimental groups. The control group and two experimental groups (20 and 120 min of seizures) were the same as the ones used for assay of metabolites in cerebral cortex, hippocampus and cerebellum (Folbergrová et al., 1981). For analysis of FFA concentrations, samples of cerebral cortex and hippocampus were obtained from the contralateral hemisphere, while the cerebellum was split on the midline. The remaining experimental group was one in which the animals were given 10 mg·kg⁻¹ of indomethacin (i.v.) 15 min prior to induction of seizures. In this group, the brain was frozen *in situ* after 20 min of seizure activity.

Series B consisted of one control group and three experimental groups. In the first two of the experimental groups, tissue was sampled after 5 and 60 min of seizures. In the third, indomethacin was given just prior to induction of seizures, and tissue freezing occurred after 60 min of seizure activity.

Since previous (unpublished) results had shown that indomethacin does not alter the tissue FFA content, no indomethacin-injected control groups were assembled. Attempts were made, though, to maintain the control animals anaesthetised for periods corresponding to these employed in bicuculline-injected animals.

Since details of results of previously published material (Chapman et al., 1980) were not given, such details are presented here (*series C*).

Animals: Operative and sampling techniques

We used male S.P.F. Wistar rats, weighing 250–400 g (Møllegaard Avlslaboratorium, Copenhagen). The animals had free access to tap water and rat pellets (Astra-Ewos, Søderlæje) until the experiment began. The operative preparations were as described in a recent publication (Folbergrová et al., 1981). In brief, the animals were anaesthetized with 2–3% halothane, tracheotomized, immobilised with tubocurarine chloride (0.5 mg·kg⁻¹), and ventilated during operation on 1% halothane in a N₂O-O₂ mixture (70:30, vol/vol). Catheters were inserted into a femoral artery and vein, EEG electrodes were inserted into the skull bone, and preparations were made for freezing the tissue *in situ* with liquid nitrogen. When the operative procedures had been completed, the halothane supply was discontinued and ventilation continued on a N₂O-O₂ mixture (70:30). A steady state period of at least 30 min was allowed before seizures were induced. During that time, body temperature was adjusted to 37°C and arterial Pco₂ to 35–40 mm Hg. Po₂ being held at 100 mm Hg, or higher. At the end of the steady state period, seizures were induced by the i.v. injection of bicuculline (1.2 mg·kg⁻¹). To minimize the initial ('ictal') increase in blood pressure, blood was withdrawn so as to reduce mean arterial blood pressure to 100–120 mm Hg just prior to the injection of bicuculline.

Body temperature, mean arterial blood pressure, arterial Po₂, Pco₂ and pH, as well as EEG activity, were measured with standard techniques (see Folbergrová et al., 1981).

Chemicals

Chloroform, toluene, diethylether, dichlorofluorescein, and silica gel 60 were from Merck, Darmstadt, West Germany; methanol, Pronalys, from May and Baker Ltd, Dagenham, England; light petroleum (b.p. 35–60°C) from Mallinckrodt Inc., Paris, Kentucky, U.S.A.; BHT (2,6-di-*tert*-butyl-*p*-cresol) from Fluka AG, Switzerland; and heneicosanoic acid (21:0) from Larodan Lipids AB, Malmö. Standards of fatty acid methyl esters were from Sedary Research Laboratories, Ontario, Canada. All chemicals and solvents were analytical or spectrographic grade. Bicuculline hydrochloride (Sigma Chemical Co.) was dissolved in 0.1 M HCl and the solution brought to pH 4–5 with 0.1 M NaOH and diluted with water to yield a concentration of 1.2 mg/ml. Indomethacin (Confortid®, Dumex) was supplied as freeze-dried powder. When dissolved in sterile water (10 mg·ml⁻¹) it gives an approximately isotonic solution with a pH of 7.7–7.8.

Extraction and analysis of brain lipids

For assaying free fatty acids we used conventional techniques, as described in detail in previous communications (Rehncrona et al., 1980; Agardh et al., 1981a). In summary, the frozen tissue samples (about 100 mg) were homogenised in at least 26 volumes of chloroform-methanol-water (1:1:0.2, by vol) with BHT added as an antioxidant. After centrifugation, we reextracted the tissue residue with chloroform-methanol-water (3:1:0.1, by vol) before filtering and combining the extracts. Heneicosanoic acid was added to the combined extracts as an internal standard. Following addition of 3 ml of 1% NaCl in 0.01 M HCl and centrifugation, the upper layer was discarded, and the lower layer washed with methanol-water (1:1, vol/vol). The chloroform phase was

then evaporated in a stream of N_2 , and the lipids were dissolved in chloroform-methanol (2:1, vol/vol). Aliquots of this solution were taken for determination of total lipid phosphorus (P), total fatty acids (FA) (see Rehnrcrona et al., 1980) and free fatty acids (FFA). For separation of the FFA fraction, we used TLC on silica gel 60 with light petroleum/diethyl ether/acetic acid (55:45:2, by vol) as solvent. The FFA band was visualized in UV light after spraying the plates with dichlorofluorescein. The FFA fraction was then scraped off the plate and the acids were esterified in 0.38 M sulphuric acid in methanol-toluene (1:1, vol/vol). We separated individual FFA methyl esters by GLC (Model 3700, Varian, Zug, Switzerland), with diethylene glycol succinate (10% on 80/100 Varaport 30) as the stationary phase and nitrogen as the carrier gas, the temperature being programmed from 160 to 185°C at a rate of 1°C·min⁻¹. The fatty acid methyl ester peaks were identified according to their retention times and the linearity of the flame ionisation detector was checked by use of commercially available mixed standards. Peak area measurements, made with an electronic integrator (model CDS 111, Varian) were used for quantitation.

Calculations

Since the results gave no clear indication that the FFA values showed a normal distribution, statistical differences were calculated by the nonparametric Mann-Whitney *U*-test (Siegel, 1956).

RESULTS

Physiological variables

Table 1 gives the physiological variables in series A and B. In the table, the two control materials were combined. In all groups, body temperature was close to 37°C, and arterial P_{O_2} was approximately 100 mm Hg. Changes in arterial blood pressure, P_{CO_2} and pH were similar to those previously described (Meldrum and Nilsson, 1976; Chapman et al., 1977). Thus, in all seizure groups blood pressure could be maintained at ~120 mm Hg. Arterial P_{CO_2} was reduced early in seizures (5 and 20 min) and later increased towards control, and pH was considerably reduced from 20 min onwards. As the results demonstrate, physiological variables were similar in the 'standard' 20- and 60-min groups and

in those in which animals were pretreated with indomethacin.

In the previous 30- and 60-min groups (Chapman et al., 1980) physiological variables were similar to those observed in the present 20- and 60-min groups. In the previous 1-min group, though, mean arterial blood pressure was increased (180 ± 5 mm Hg) and neither the P_{aCO_2} (38.1 ± 1.4 mm Hg) nor the pH (7.42 ± 0.01) was reduced below control.

In each animal included in the series to be discussed below, the bicuculline injection gave rise to the expected EEG changes (see Meldrum and Nilsson, 1976; Chapman et al., 1977), and the seizure discharge continued unabated to the end of the experiment. Pretreatment of the animals with indomethacin did not influence the seizure discharge.

Tissue fatty acid and lipid phosphorus contents

The concentrations of total and individual phospholipid-bound fatty acids and phosphorus measured (not shown) were in agreement with previous results for control animals from our laboratory (see Agardh et al., 1981a; Rehnrcrona et al., 1982). There were no significant changes in values obtained in any of the experimental groups compared with the controls.

Total and individual free fatty acids

Values obtained for total and individual FFA in the cerebral cortex of bicuculline-injected animals (standard and indomethacin-treated) are given in Table 2 together with the appropriate control values. One grossly aberrant FFA value was excluded from the control group of series B. The three control groups showed similar values for total FFA content as well as for 16:0 and 20:4 contents. In series B, the content of 18:0 was higher and that of 18:1 lower ($p < 0.05$) than the combined values for series A and C. For that reason, we evaluated time-related changes in individual fatty acids (see below) by calculating each value measured in bicuculline-injected animals as a percentage of the appropriate control values. Estimation of changes in 22:6 pre-

TABLE 1. Physiological variables in control rats and in animals with bicuculline-induced seizures

Duration of seizures (no. of animals)	Temperature (°C)	MABP (mm Hg)	P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)	pH
Control (12)	36.9 ± 0.1	161 ± 3	103 ± 2	37 ± 2	7.40 ± 0.01
5 min (6)	36.6 ± 0.2	123 ± 2	98 ± 3	28 ± 2	7.37 ± 0.03
20 min (6)	36.4 ± 0.1	120 ± 1	116 ± 2	28 ± 2	7.08 ± 0.02
60 min (6)	37.3 ± 0.3	122 ± 1	101 ± 4	34 ± 2	6.93 ± 0.05
120 min (6)	37.1 ± 0.2	115 ± 3	106 ± 2	33 ± 1	7.11 ± 0.04
20 min (6) ^a	36.5 ± 0.1	124 ± 5	110 ± 3	34 ± 2	7.03 ± 0.20
60 min (6) ^a	37.2 ± 0.1	118 ± 1	102 ± 5	37 ± 1	6.92 ± 0.04

The values are means ± SEM.

^a Animals pretreated with indomethacin (10 mg·kg⁻¹) prior to induction of seizures.

BRAIN FREE FATTY ACIDS DURING SEIZURES

TABLE 2. Total and individual FFA concentrations in the cerebral cortex during bicuculline-induced seizures

Series	Duration of seizures in min (n)	Total FFA ($\mu\text{mol} \cdot \text{g}^{-1}$)	16:0 ($\mu\text{mol} \cdot \text{g}^{-1}$)	18:0 ($\mu\text{mol} \cdot \text{g}^{-1}$)	18:1 ($\mu\text{mol} \cdot \text{g}^{-1}$)	20:4 ($\mu\text{mol} \cdot \text{g}^{-1}$)	22:6 ($\mu\text{mol} \cdot \text{g}^{-1}$)
A	Control (6)	0.166 $\pm$ 0.008	0.076 $\pm$ 0.005	0.049 $\pm$ 0.002	0.029 $\pm$ 0.002	0.010 $\pm$ 0.002	0.001 ^a
	20 min (6)	0.439 $\pm$ 0.042 ^d	0.110 $\pm$ 0.015 ^c	0.204 $\pm$ 0.019 ^d	0.047 $\pm$ 0.006 ^d	0.069 $\pm$ 0.006 ^d	0.008 $\pm$ 0.001 ^d
	20 min (6) ^b	0.381 $\pm$ 0.030	0.098 $\pm$ 0.007	0.176 $\pm$ 0.016	0.045 $\pm$ 0.003	0.056 $\pm$ 0.007	0.007 $\pm$ 0.001
	120 min (6)	0.216 $\pm$ 0.017 ^c	0.086 $\pm$ 0.006	0.068 $\pm$ 0.006 ^c	0.037 $\pm$ 0.003 ^c	0.019 $\pm$ 0.003 ^c	0.007 $\pm$ 0.001 ^d
B	Control (5)	0.171 $\pm$ 0.007	0.069 $\pm$ 0.002	0.072 $\pm$ 0.004	0.021 $\pm$ 0.002	0.008 $\pm$ 0.002	0.0004 ^a
	5 min (6)	0.399 $\pm$ 0.018 ^d	0.096 $\pm$ 0.007 ^c	0.193 $\pm$ 0.015 ^d	0.034 $\pm$ 0.003 ^d	0.070 $\pm$ 0.003 ^d	0.006 $\pm$ 0.001 ^d
	60 min (6)	0.262 $\pm$ 0.034 ^d	0.093 $\pm$ 0.012	0.112 $\pm$ 0.013 ^d	0.032 $\pm$ 0.005	0.020 $\pm$ 0.006 ^c	0.005 $\pm$ 0.003
	60 min (6) ^b	0.213 $\pm$ 0.019	0.078 $\pm$ 0.010	0.091 $\pm$ 0.005	0.029 $\pm$ 0.006	0.013 $\pm$ 0.004	—
C	Control (6)	0.162 $\pm$ 0.005	0.071 $\pm$ 0.010	0.055 $\pm$ 0.005	0.029 $\pm$ 0.004	0.006 $\pm$ 0.0004	0.002 ^a
	1 min (5)	0.365 $\pm$ 0.042 ^d	0.082 $\pm$ 0.008	0.154 $\pm$ 0.019	0.043 $\pm$ 0.008	0.078 $\pm$ 0.007 ^d	0.008 $\pm$ 0.003
	30 min (6)	0.412 $\pm$ 0.041 ^d	0.109 $\pm$ 0.015	0.205 $\pm$ 0.016	0.040 $\pm$ 0.006	0.046 $\pm$ 0.00 ^d	0.512 $\pm$ 0.002 ^d
	60 min (6)	0.229 $\pm$ 0.015	0.080 $\pm$ 0.005	0.086 $\pm$ 0.008	0.036 $\pm$ 0.003	0.018 $\pm$ 0.002 ^d	0.008 $\pm$ 0.001 ^d

Values are means $\pm$ SEM.

^a In each of the three control groups, measurable amounts of 22:6 were found in only two animals. The data of series C are from Chapman et al. (1980).

^b These animals were injected with indomethacin (10 mg $\cdot$ kg⁻¹) prior to induction of seizures.

^{c,d} Statistical significance by the Mann-Whitney U-test: c, $p < 0.05$; d, $p < 0.01$.

sents problems, since in each of the three series, only two brains gave measurable amounts of this acid.

Three main results emerge from the data of Table 2. First, the onset of seizures was accompanied by rapid accumulation of FFA, with a peak increase at 5–20 min. Second, although the FFA concentrations then decreased, a significant elevation of total FFA and arachidonic acid concentration persisted after 60 and 120 min. Third, the rapid fall in arachidonic acid concentration, following its massive accumulation at 1–5 min, was not influenced by indomethacin.

We used the data of Table 2 to analyse further the time course of changes in total and individual FFAs. In doing so, we combined the values for the two 60-min groups. Furthermore, since the 1-, 20- and 30-min groups each contained one grossly aberrant value for 16:0, 18:0 or 18:1 (being 10–20 SEs above the mean of the remaining five), these animals were excluded from the comparison.

Figure 1 shows that peak FFA concentrations were obtained after 5 min of seizure activity. The total FFA content then remained stable for at least 15 min. The difference in FFA content between the 20- and 30-min groups was not statistically significant. A highly significant decrease was observed after 60 min, with little further change in the subsequent 60-min period.

Time-dependent changes in individual FFA are illustrated in Fig. 2. Apart from combining the 60-min groups we also combined the 20- and 30-min groups since all values in these groups were similar ('25-min' group). The results allow the following conclusions. First, although the seizure activity was accompanied by some increase in the concentrations of 16:0 and 18:1, the increases in 18:0 and,

especially, of 20:4 were much more marked. Second, the remaining elevation of total FFA content at 60 and 120 min was due to increases in all acids measured. Third, whereas the concentrations of 16:0, 18:0 and 18:1 either remained constant or rose in the 1–25-min period, the arachidonic acid concentration fell gradually after 1 min. This rapid, secondary decrease in arachidonic acid concentration was 'selective'. If we can assume a normal 22:6 concentration of about 0.001 $\mu\text{mol} \cdot \text{g}^{-1}$ (see Table 2) we must conclude that the concentration of 22:6 remained increased by a factor of 5 or more throughout the whole seizure period.

Changes in free fatty acid concentrations in the hippocampus and cerebellum

Total and individual FFA concentrations in these structures were measured after 20 and 120 min in

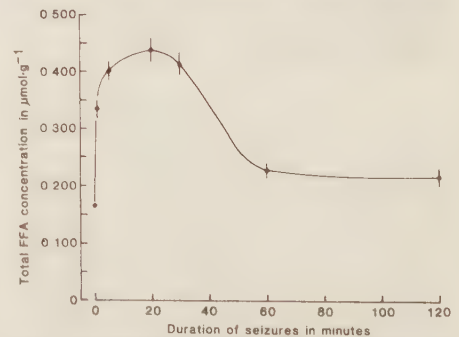


FIG. 1. Time-dependent changes in total FFA content in the cerebral cortex during continuous bicuculline seizures of 1–120 min duration. The values are means $\pm$ SEM.

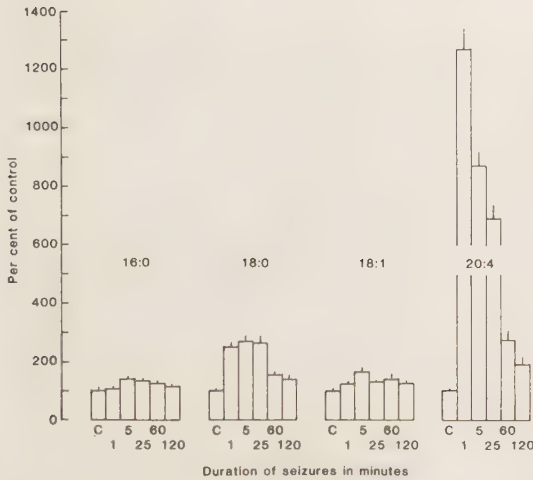


FIG. 2. Time-dependent changes in the cerebral cortical concentrations of individual FFAs (16:0, 18:0, 18:1, and 20:4) during continuous bicuculline-induced seizures of 1–120 min duration. The '25-min' group was obtained by pooling data from the 20- and 30-min groups.

series A (Table 3). The results indicate that the FFA pool, and the concentrations of polyenoic fatty acids were slightly higher in the hippocampus than in the cerebral cortex (cf. Table 2). After 20 min of seizures, the changes in total and individual FFA were similar in the two structures. Results obtained in indomethacin-treated animals (20 min of seizures) were identical (data not shown). For example, total FFA content was 0.502 ± 0.021 and arachidonic acid concentration $0.071 \pm 0.006 \mu\text{mol} \cdot \text{g}^{-1}$. After 120 min, only the 16:0, 18:0 and 20:4 concentrations remained significantly elevated above control in the hippocampus. The results suggest, though, that changes in the hippocampus and the cerebral cortex were similar (see absolute values for total FFA content and docosahexaenoic acid concentration).

A comparison with data presented in Table 2 (see above) demonstrates that the cerebellum had a lower concentration of 20:4, and a higher concentration of 22:6, than the cerebral cortex, but the

total FFA content was similar. Seizure-induced changes in FFA concentrations in the cerebellum were quite different from these in the cerebral cortex and hippocampus. Thus, after 20 min the only significant change in the cerebellum was a sevenfold increase in arachidonic acid concentration; after 120 min, the FFA pattern was not different from control. Results obtained in indomethacin-injected animals were very similar to those of the standard 20-min group: the total FFA content ($0.202 \pm 0.030 \mu\text{mol} \cdot \text{g}^{-1}$) was not significantly different from control, and the concentration of 20:4 was $0.015 \pm 0.005 \mu\text{mol} \cdot \text{g}^{-1}$.

DISCUSSION

We will focus the discussion of the results presented on the three questions posed in the introduction.

TABLE 3. Total and individual FFA concentrations in the hippocampus and cerebellum during bicuculline-induced seizures

Sample	Duration of seizures (n)	Total FFA ($\mu\text{mol} \cdot \text{g}^{-1}$)	16:0 ($\mu\text{mol} \cdot \text{g}^{-1}$)	18:0 ($\mu\text{mol} \cdot \text{g}^{-1}$)	18:1 ($\mu\text{mol} \cdot \text{g}^{-1}$)	20:4 ($\mu\text{mol} \cdot \text{g}^{-1}$)	22:6 ($\mu\text{mol} \cdot \text{g}^{-1}$)
Hippocampus	Control (6)	0.199 ± 0.025	0.084 ± 0.011	0.065 ± 0.007	0.033 ± 0.004	0.014 ± 0.001	0.004 ± 0.001^a
	20 min (5)	0.529 ± 0.034^c	0.148 ± 0.010^c	0.230 ± 0.012^c	0.063 ± 0.008^c	0.077 ± 0.004^c	0.011 ± 0.002^c
	120 min (5)	0.291 ± 0.022	0.113 ± 0.008^b	0.099 ± 0.008^b	0.043 ± 0.005	0.028 ± 0.007^b	0.008 ± 0.002
Cerebellum	Control (6)	0.143 ± 0.013	0.064 ± 0.006	0.045 ± 0.005	0.028 ± 0.004	0.002 ± 0.000	0.004 ± 0.001^a
	20 min (6)	0.171 ± 0.016	0.061 ± 0.004	0.063 ± 0.007	0.027 ± 0.002	0.015 ± 0.003^c	0.006 ± 0.001
	120 min (4)	0.113 ± 0.015	0.049 ± 0.005	0.033 ± 0.005	0.024 ± 0.003	0.002 ± 0.000	0.006 ± 0.002

Values are means $\pm$ SEM.

^a In these groups, five of six animals had detectable amounts of 22:6.

^{b,c} Statistical significance: b, $p < 0.05$; c, $p < 0.01$.

Time course of free fatty acid changes in the cerebral cortex

The present results (see Fig. 1) demonstrate that the total FFA content increases during the first 5 min of seizure discharge, remains unchanged for an additional 15–25-min period, and then decreases to values only moderately raised above control. This secondary fall in FFA concentrations is not related to a corresponding change in cerebral energy state (Chapman et al., 1977), nor is it due to attenuation of the epileptic discharge or to a secondary reduction in cerebral metabolic rate (Meldrum and Nilsson, 1976; Ingvar and Siesjö, unpublished results). Previous results (Bazán, 1971; Marion and Wolfe, 1978), and the present ones, suggest that the fatty acids released mainly emanate from phospholipids with a high 18:0 and 20:4 content, e.g. phosphatidylinositol (see Sun et al., 1979). Thus, although it is commonly assumed that increased intracellular Ca^{2+} activity is at least partly responsible for deacylation of phospholipids, increased membrane turnover due to release of receptor agonists could be a contributing factor (see Michell, 1975; Hawthorne and Pickard, 1979; Hirata and Axelrod, 1980). It remains to be shown whether the secondary reduction in FFA content is due to reduced Ca^{2+} activation or to reduced membrane turnover. In this context, we wish to recall that the reduction in tissue NA content occurs during the first 30 min of seizures (Calderini et al., 1978), and that massive accumulation of adenosine is a transient event (see Siesjö et al., 1982).

Influence of indomethacin on arachidonic acid disappearance

During severe hypoglycemia, an increased total tissue FFA content is upheld for at least 60 min but, following its massive accumulation at 5 min, arachidonic acid falls in concentration (Agardh et al., 1981a,b). In the present experiments, a secondary reduction in arachidonic acid occurred at a time when concentrations of other acids either rose or remained constant. The combined results suggest that, in pathological situations with maintained circulation and oxygen supply, arachidonic acid is either preferentially reacylated, or metabolised in reactions for which the acid is a substrate. Thus, although evidence exists that fatty acid-CoA ligases prefer 20:4 to 18:0 or, alternatively, that a special acylation system exists for arachidonic acid (Sun et al., 1979), one cannot dismiss the possibility that at least part of the reduction in arachidonic acid concentration was due to oxidation by cyclo-oxygenase. It has previously been shown that indomethacin virtually blocks the production of prostaglandins that otherwise occurs in the reoxygenation period following ischemia (Gaudet et al., 1980). In the present experiments, indomethacin failed to affect the rate of disappearance of arachidonic acid.

Therefore, unless this decrease is entirely due to reacylation, alternative pathways of degradation must be explored. At least in theory, these could involve oxidation by lipoxygenases (see Murphy et al., 1979; Samuelsson, 1980) or peroxidation initiated by free radical reactions (e.g. Pryor, 1978; Chan and Fishman, 1980).

Regional differences in free fatty acid accumulation

The present results demonstrate that FFA changes in the hippocampus during seizures were similar to those observed in the cerebral cortex, and that those affecting the cerebellum were distinctly different. Thus, in the latter structure the only significant change from the normal was a 5–10-fold increase in arachidonic acid concentration from control values at 20 min. Since the control values were low, the 20-min values for 20:4 were only about 20% of these observed in the other two structures. Probably this modest alteration of FFA concentrations reflects the fact that cerebellar metabolism is only moderately perturbed (Folbergrová et al., 1981), this in turn being a reflection of a low metabolic rate (Siesjö and Abdul-Rahman, 1979; Ingvar and Siesjö, 1982). We note that the two 'vulnerable' structures (cerebral cortex and hippocampus) show a marked accumulation of FFA whereas the 'resistant' one (cerebellum) does not. Whether a causal relationship exists between FFA accumulation and cell damage is at present a matter of speculation (see Siesjö, 1981).

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INFLUENCE OF LESIONS OF THE NORADRENERGIC LOCUS

COERULEUS SYSTEM ON THE CEREBRAL METABOLIC

RESPONSE TO BICUCULLINE-INDUCED SEIZURES

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ABSTRACT

The objective of the present study was to explore if lesions of the ascending noradrenergic pathways, originating in the locus coeruleus, modulate the cerebral metabolic response to bicuculline-induced seizures in rats. Bilateral noradrenergic lesions were performed by 6-hydroxydopamine injections in the caudal mesencephalon, 12-24 days before seizures were induced in animals ventilated on N₂O:O₂ (75:25). After 5 min of seizures the brain was frozen in situ and cerebral cortex and hippocampus were sampled for analysis. Labile phosphates, glycolytic metabolites, cyclic nucleotides, and free fatty acids were measured. In another series, lesioned animals were used for measurements of cerebral oxygen consumption.

The noradrenergic lesions neither modified the electroencephalographically recorded seizure discharge, nor did they alter cerebral oxygen consumption or cerebral energy state. However, when compared to sham-operated animals, those with noradrenergic lesions had significantly higher (115% and 68%) glycogen concentrations and lower (50% and 52%) cyclic AMP concentrations in cerebral cortex and hippocampus, respectively, demonstrating the marked influence of noradrenergic activity on adenylate cyclase activity and glycogenolysis. The lesions failed to modulate the rise in free fatty acids in the cerebral cortex, or the cyclic GMP concentrations in the cerebral cortex and hippocampus. Thus, increased noradrenergic activity during status epilepticus does not seem responsible for lipolysis or for activation of guanylate cyclase.

INTRODUCTION

Evidence is now accumulating that noradrenaline (NA) exerts inhibitory actions at central synapses, and that this action is mediated via the second messenger cAMP. Most of this evidence is based on electrophysiological recordings of cell activity following iontophoretic application of the neurotransmitter and/or the messenger nucleotide in normal animals (for literature see 3,31). In recent years it has become increasingly clear that the intrinsic noradrenergic system of the brain also modulates epileptic activity induced by electrical stimulation or convulsant drugs. Chemical and/or surgical lesions of central noradrenergic pathways have been shown to facilitate convulsive activity induced by electroshock or kindling

(9,23,24). Furthermore, pretreatment of animals with reserpine or propranolol aggravated the seizures induced by pentylenetetrazol, and attenuated or blocked the rise in cAMP (16). Finally, application of β -adrenergic agonists or activation of adrenergic afferents to transplants of rat hippocampus into the anterior chamber of the eye of rats was found to inhibit penicillin induced seizure activity (15). All these results suggest that epileptiform activity is modulated by NA, which exerts a cAMP-mediated inhibitory effect.

It has been shown that seizure activity induced by electrical stimulation, or a variety of convulsant drugs, leads to increased brain tissue concentrations of cAMP and cGMP (10,13,14,22). In view of the results quoted above, it is tempting to conclude that the increase in cAMP is related to enhanced release of NA at central synapses, and its action on β -adrenergic receptors . Supporting evidence was obtained by results showing that bicuculline induced seizures are accompanied by an increased activity in central noradrenergic neurons (5). However in view of the fact that adenylate cyclases in the brain are stimulated by adenosine as well (for review, see 18), and that aminophylline facilitates seizures with an accompanying attenuation of cAMP accumulation (16), the quantitative role played by NA in adenylate cyclase activation remains unclear. A further difficulty arises from the fact that NA has been reported to cause activation of cerebral guanylate cyclases, with a resulting rise in cGMP (17,26).

It is clear that if NA suppresses neuronal activity, an increased noradrenergic activity during seizures could secondarily reduce overall metabolic rate. However, a more direct metabolic effect of such activity may arise from the proposed coupling between increased cAMP concentration and phosphorylase activity. Thus, enhanced phosphorylase activity with a resulting breakdown of glycogen has been noted after catecholamine administration (8) , as well as during seizures induced by electroshock and various convulsant drugs (11,12,22). It seems reasonable to assume that glycogenolysis is triggered by cAMP, mediating activation of phosphorylase b kinase with a resulting conversion of phosphorylase b to a. However at least under some conditions a dissociation occurs between accumulation of cAMP and phosphorylase activation, suggesting that glycogenolysis occurs by other mechanisms as well, e.g. by cellular influx of calcium (see 14)

The nucleus locus coeruleus, which is the only source for nor-adrenergic innervation of the neocortex and hippocampus (20), has been shown to partly regulate cAMP levels in the normal rat (19,29). In the present study we explored whether lesions to the ascending NA system from the locus coeruleus modulate the cerebral metabolic response to bicuculline-induced seizures, particularly the concentrations of glycogen and cyclic nucleotides. In order to explore whether any such modulation was secondary to changes in overall metabolic rate, cerebral energy state and cerebral metabolic rate for oxygen were measured as well. In addition, since release of NA has been assumed to cause activation of phospholipases (1), we also measured free fatty acids in lesioned and sham-operated animals subjected to seizure activity.

MATERIALS AND METHODS

1. Animals and experimental groups

The experiments were performed on male Wistar rats weighing between 350 and 420 g. The animals had free access to food (San bolagen, Malmö, Sweden) and water until the time of operation.

There were two series of animals. In the first, the animals were subjected to bilateral lesions of the noradrenergic system innervating the forebrain, or were sham operated. Twelve to twenty two days later, the animals were reoperated and the effect of 5 min of bicuculline-induced seizures on labile phosphates, glycolytic metabolites, and cyclic nucleotides in the cerebral cortex and the hippocampus were studied (n=6 for both lesioned and sham-operated groups). FFA was measured in the parietal cortex from the hemisphere not used for analysis of other metabolites. Since samples for FFA analysis were not obtained in all lesioned animals, two additional animals were studied. In all animals included in the series, analyses of NA content showed that lesions were efficient (see below).

In the second series, devoted to measurements of cerebral blood flow (CBF) and metabolic rate for oxygen ($CMRO_2$), two groups were subjected to 5 min of bicuculline seizures following NA lesions or sham operation at least 12 days earlier.

2. Operative and sampling techniques

Lesion techniques

Lesions were performed under barbiturate anesthesia (Brietal, Lilly, 40 mg·kg⁻¹ i.p.) with the animal placed in a stereotaxic

instrument (Kopf instrument, Tujunga, Ca, USA). Bilateral lesions of the ascending noradrenaline fibers from pons and medulla oblongata were made at the level of the caudal mesencephalon, rostral to the catecholamine-containing cells in the locus coeruleus and subcoeruleus area (for details see 7). The lesions were produced by injections of 6-hydroxydopamine (8 μ g in 4 μ l of saline on each side) close to the dorsal tegmental catecholamine bundle. In sham-operated animals the cannula was lowered to the same coordinates but no injection of 6-hydroxydopamine was made. All further experiments were performed 12 to 22 days after the lesions had been carried out.

Procedures for measuring tissue metabolites

The animals were anesthetized in a jar with a mixture of 3% halothane and 75% N₂O in oxygen. When unresponsive, the animals were tracheostomized, paralyzed with tubocurarine (1.5mg·kg⁻¹) and connected to a Starling type respirator. The ventilator was set to yield a PaCO₂ of between 35 and 40 mmHg prior to induction of seizures. Blood gases were evaluated using samples of 150 μ l (Eschweiler and Co., Kiel, and Radiometer, Copenhagen). Rectal temperature was maintained close to 37 °C with a heating lamp. The halothane concentration was then reduced to 0.7% until the operation was completed. The femoral vessels were cannulated for blood pressure recording, blood sampling, and infusion of drugs. The bone of the skull was exposed through a skin incision. In all experiments two gold plated EEG screws were inserted in the bone in the parietal region and the EEG was recorded continuously (Siemens Elema, Stockholm). A plastic funnel was placed in a skin incision over the exposed skull bone, making it possible to later freeze the brain in situ by pouring liquid nitrogen into the funnel. The animals were heparinized (10 IU i.v.) and allowed to recover for at least 30 min before induction of seizures. Bicuculline (Sigma) was then injected i.v. (1.2mg/kg) following a lowering of the blood pressure of the animal to approximately 100 mmHg by bleeding of 3-5 ml. For details of procedures, see (14).

Tissue metabolites were determined in the parietal cortex and in the hippocampus after a seizure period of 5 min. For dissection of tissue samples, the brains (stored in liquid nitrogen) were allowed to reach a temperature of -22 °C. One hemisphere was used for analysis of labile phosphates, glycolytic metabolites, and cyclic nucleotides (25-30 mg of parietal cortex and an equally large sample from the

hippocampus) while the other was used for analysis of cortical free fatty acids (70-90 mg of parietal cortex). Samples (5-15 mg) for measurement of NA content were taken from the frontal poles and from the ipsilateral hippocampus.

Procedures for measuring CBF and CMRO₂

With two exceptions, anesthetic and operative procedures were identical. First, a short brachial catheter was inserted for sampling of arterial blood. Second, a burr hole was placed over the superior sagittal sinus, allowing sampling of cerebral venous blood. Both frontal poles (10-25 mg) were taken for later analysis of NA content.

3. Methods and analytical techniques

Labile phosphates, glycolytic metabolites and cyclic nucleotides

Samples of cerebral cortex and hippocampus were extracted with HCl-methanol at -22°C, and subsequently with perchloric acid at 0°C. Tissue contents of glycogen, glucose, pyruvate, lactate, phosphocreatine (PCr), ATP, ADP, and AMP were measured with enzymatic, fluorometric techniques (for information on analytical conditions, see 14). A protein binding technique was used to measure cAMP. For measuring cGMP we used the radioimmunoassay technique. However, for this metabolite an acidic extract (pH 6.2) was prepared (21).

Noradrenaline

NA was determined using a radioenzymatic method on an acidic extract. Details of the procedure has been given in a previous communication (28).

Free fatty acids

For assaying free fatty acids we used conventional techniques, as described in detail in previous communications (27). In summary, the frozen tissue samples (about 100 mg) were homogenized in at least 26 volumes of chloroform-methanol-water (1:1:0.2, v/v) with BHT (2,6-ditert-butyl-p-cresol) added as an antioxidant. After further processing of the samples, the FFA fraction was separated on silica gel 60 with light petroleum:diethyl ether:acetic acid (55:45:2 v/v) as solvents. The FFA band was visualized in UV-light and scraped off the plates, the FFA were then esterified, and the methylesters separated by GLC and quantified with an electronic integrator. Heinecosanoic acid was added to the extract as an internal standard. The fatty acid methyl ester peaks were identified according to their retention

times. The linearity of the flame ionization detector was checked with commercially available mixed standards.

CBF-CMRO₂

We measured CBF with a modification of the Kety-Schmidt technique, using ¹³³Xenon as the inert tracer (2). The measurements were started after 5 minutes of seizure activity. Just before and during the measurement four sets of arterial and venous samples were taken for estimation of the arteriovenous differences in oxygen concentration (AVDO₂). The cerebral metabolic rate for oxygen (CMRO₂) was subsequently calculated as the product of the CBF times the AVDO₂. Experiments were omitted if AVDO₂ prior to and during flow measurement varied by more than 10%. At the end of the flow measurements both frontal poles were frozen for later analysis of NA content.

Calculations:

Statistical differences were evaluated using Student's t-test.

RESULTS

In all animals, administration of bicuculline gave rise to the expected seizure discharge and after 5 min, the EEG showed a continuous burst suppression pattern (see 25). No indication was obtained that the NA lesions affected the pattern of epileptic discharge (see Discussion).

Physiological parameters, as measured prior to freezing of the brain in situ, or during the CBF-CMRO₂ measurements, are shown in table 1. The data demonstrate that variations in temperature, blood pressure, blood gases, and pH could not have influenced the

Table 1. Physiological parameters in the different experimental groups.

Experimental groups	T C°	MABP mmHg	PO ₂ mmHg	PCO ₂ mmHg	pH
Sham-operated	36.5±0.1	122±2	106±3	29.4±1.4	7.39±0.03
Lesioned, metabolites	36.3±0.1	121±2	95±3	31.5±3.3	7.38±0.04
Lesioned, FFA	36.5±0.1	119±2	101±5	32.8±2.7	7.37±0.03
Shamoperated CBF-CMRO ₂	36.9±0.2	131±7	114±2	28.4±2.3	7.26±0.04
Lesioned CBF-CMRO ₂	36.4±0.3	129±5	107±5	27.2±3.2	7.26±0.04

Values are given as MEAN±S.E. n=6 in all groups.
MABP=mean arterial blood pressure.

differences in results between lesioned and sham-operated animals.

Labile phosphates, glycolytic metabolites and cyclic
nucleotides

The concentrations of labile metabolites, including cyclic nucleotides, and of NA are given in table 2. As can be seen, the

Table 2. Energy metabolites in cortex and hippocampus in rats, with lesions of the ascending noradrenergic pathways or sham-lesions, subjected to five minutes of status epilepticus.

	Cortex		Hippocampus	
	lesioned	sham	lesioned	sham
Phosphocreatine	3.27 $\pm$.17	3.27 $\pm$.15	3.57 $\pm$.31	3.50 $\pm$.07
ATP	2.32 $\pm$.04	2.27 $\pm$.05	2.54 $\pm$.19	2.32 $\pm$.04
ADP	.27 $\pm$.03	.26 $\pm$.01	.30 $\pm$.02	.31 $\pm$.02
AMP	.05 $\pm$.00	.05 $\pm$.00	.05 $\pm$.00	.06 $\pm$.01
Glucose	.72 $\pm$.05	.57 $\pm$.11	.63 $\pm$.08	.58 $\pm$.07
Glycogen	2.16 $\pm$.27 **	1.00 $\pm$.15	1.69 $\pm$.21 *	1.01 $\pm$.14
Lactate	7.41 $\pm$.43	7.11 $\pm$.30	8.06 $\pm$.60	6.88 $\pm$.38
Pyruvate	.15 $\pm$.01	.15 $\pm$.01	.16 $\pm$.02	.15 $\pm$.01
Lac/Pyruv	49.9 $\pm$ 2.7	47.2 $\pm$ 1.6	54.9 $\pm$ 7.4	48.2 $\pm$ 3.0
cAMP	2.54 $\pm$.23 **	5.03 $\pm$.49	1.24 $\pm$.07 **	2.59 $\pm$.30
cGMP	-	-	.300 $\pm$.019	.307 $\pm$.025
NA content	.010 $\pm$.003 ***	.186 $\pm$.001	.011 $\pm$.005***	.255 $\pm$.038

Values are given as Mean $\pm$ S.E. Unit is μ mol/g brain tissue wet weight, except NA content which is given in ng/g

* p<0.05, **p<0.01, and ***<0.001 between sham-operated and lesioned side

lesions reduced the NA content of cortex and hippocampus to about 5% of control.

Concentrations of labile metabolites in the cerebral cortex and the hippocampus measured in sham-operated animals were similar to those previously measured in "normal" animals subjected to 5 or 20 min of seizures (6,14).In summary, seizure-induced changes include a moderate perturbation of cerebral energy state, reductions in glycogen and glucose concentrations, and increases in the tissue concentrations of lactate (with a raised lactate/pyruvate ratio) and cyclic nucleotides.

As the results of table 2 demonstrate, the noradrenergic lesions failed to alter the seizure-induced changes in labile organic phosphates, or in glucose, lactate, and pyruvate concentrations. However the lesions significantly reduced the decrease in glycogen

concentration and the increase in cAMP concentration in both the cerebral cortex and the hippocampus. Thus, when compared to sham-operated animals, those with noradrenergic lesions had 115% and 68% higher glycogen concentrations, and 50% and 52% lower cAMP concentrations in cerebral cortex and hippocampus, respectively. No change in cGMP concentration was noted in the lesioned animals. (The

Table 3. Major free fatty acids after five min of epileptic activity in cerebral cortex in rats with lesions of the ascending noradrenergic pathways or sham lesions.

	lesion n=6	Sham n=6
16:0	.123+.006	.139+.013
18:0	.222+.007	.220+.008
18:1	.064+.007	.053+.003
20:4	.110+.020	.075+.004
22:6	.009+.001	.012+.003
Total FFA	.528+.027	.499+.025
NA content	.007+.002	.196+.009

Values are given as Mean+S.E. Unit is $\mu\text{mol/g}$ brain tissue wet weight, except NA content which is given in ng/g

Table 4. CBF and metabolic rate in controls, sham-operated animals and rats with lesions of the ascending noradrenergic pathways. The two operated groups were in status epilepticus that had been started with bicuculline 5 min prior to the start of the measurement.

	Sham n=6	Lesioned n=5
CBF ml/g/min	2.61+.25	2.11+.08
AVDO ₂ $\mu\text{mol/ml}$	2.59+.21	3.19+.26
CMRO ₂ $\mu\text{mol/g/min}$	6.50+.25	6.29+.24
NA content ng/g	.184+.015	.008+.006

No differences were observed between sham-operated and lesioned animals in CBF nor CMRO₂.

analyses of cortical cGMP contents were discarded since the cGMP kit gave insufficient binding. However, later re-analysis on stored extract showed similar values in sham-operated and lesioned groups).

FFA concentrations in the cerebral cortex in lesioned and sham-operated animals are given in table 3. Also in this material, the noradrenergic lesions reduced the tissue NA content to 5% of control. As the result demonstrate, the lesions had no effects on total or individual FFA concentrations. The mean arachidonic acid concentration was somewhat higher in the lesioned group ($0.1 < p < 0.05$). The results seem to exclude that lipolysis is enhanced by increased NA activity (see Discussion).

CBF and CMRO₂

Neither CBF nor CMRO₂ differed between lesioned and sham-operated groups,

although the lesions reduced the tissue NA content to 4% of control (Table 4).

DISCUSSION

The results of the present study can be summarized as follows. When rats were subjected to bilateral 6-hydroxydopamine lesions of the ascending noradrenergic projection from the nucleus locus coeruleus, a procedure which reduced the NA content of cerebral cortex and hippocampus to 5% of control, the seizure discharge elicited by i.v. bicuculline was not altered, nor were the changes in the tissue concentrations of labile phosphates or lactate. However, the lesions significantly reduced the decrease in glycogen and the increase in cAMP concentrations which normally occur during seizure discharge. The results strongly suggest, therefore, that the enhanced activity of the central noradrenergic neurons during seizures (see 5) is responsible for a substantial part of the rise in cAMP concentration and of the glycogenolysis. Since the lesions failed to modify tissue FFA and cGMP concentrations we may also conclude that increased noradrenergic activity does not contribute to lipolysis or to stimulation of guanylate cyclase. We will discuss some aspects of these results.

1. Seizures and metabolic rate

Bicuculline-induced seizures in the rat lead to 2- to 4- fold increases in CBF (25) and to a 2- to 3-fold increase in cerebral oxygen consumption and glucose utilization (4,25). The excessive increase in metabolic rate is accompanied by moderate reductions in tissue phosphocreatine concentration and adenylate energy charge, and by an increase in lactate concentration to 5-10 $\mu\text{mol}\cdot\text{g}^{-1}$ (6,14). The seizure activity also reduces glycogen concentration, and raises the concentrations of cAMP and cGMP, and FFA (6,30)

Since previously reported results were consistent, we abstained from studying metabolites in sham-operated control animals. More important, no differences in cerebral metabolic changes were noted between sham-operated and lesioned animals. These findings, and the lack of effect of the lesions on the pattern of seizure discharge or CMRO_2 , indicate that the lesions neither affected seizure activity nor the accompanying perturbation of cerebral energy state. The findings are consistent with previous ones that although catecholaminergic lesions influence seizure thresholds and seizure propagation, they do not affect the intensity of the seizure discharge, once elicited (23).

2. Noradrenergic lesions and tissue metabolites

Two findings provide the immediate background to the present study. First, stimulation of the locus coeruleus system leads to an increase of the cAMP concentration of the brain (19,29). Second, bicuculline-induced seizures are associated with increased activity of noradrenergic neurons in the brain (5). From these results, one could expect that interruption of the noradrenergic innervation of cerebral cortex and hippocampus should attenuate the changes in cAMP and, if cAMP is a major modulator of phosphorylase activity, also in glycogen concentration. This expectation is strengthened by results showing that drugs like reserpine and propranolol reduce seizure-induced increases in cAMP (16). The present results substantiate the assumption since they demonstrate that a major part of the seizure-induced changes in cAMP and glycogen is absent in animals with lesions of the ascending noradrenergic pathways. We cannot, though, accurately estimate the role played by other modulators of adenylate cyclase, e.g. adenosine, and of phosphorylase, e.g. Ca^{2+} .

The present results demonstrate that the noradrenergic lesions failed to modulate the seizure-induced changes in FFA and cGMP concentrations. Thus we fail in this study to support the proposal of a major influence from the central noradrenergic system on the activation of phospholipases.

In conclusion, the present study has provided new information on the influence of the noradrenergic system, originating in the nucleus locus coeruleus, on cerebral metabolic events during generalized seizures. Since this influence was confined to the adenylate cyclase and the phosphorylase systems, the results amply corroborate previous data obtained on non-epileptic animals, suggesting that the central noradrenergic system, innervating the cerebral cortex and the hippocampus, mediates inhibition via an effect on cyclic AMP and facilitates mobilization of substrate via an effect of cyclic AMP on the phosphorylase complex.

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Local cerebral glucose consumption in the artificially ventilated rat: influence of nitrous oxide analgesia and of phenobarbital anesthesia

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INGVAR, M., ABDUL-RAHMAN, A. & SIESJÖ, B. K.: Local cerebral glucose consumption in the artificially ventilated rat: influence of nitrous oxide analgesia and of phenobarbital anesthesia. *Acta Physiol Scand* 1980, 109: 177–185. Received 15 Oct. 1979. ISSN 0001-6772. Laboratory for Experimental Brain Research, and Department of Neurosurgery, University of Lund, Sweden.

The objectives of the present study, which concerns local glucose consumption (1-CMR_{gl}) in the rat brain as measured with the ^{14}C -deoxyglucose technique of Sokoloff et al. (1977), were (1) to provide data for 1-CMR_{gl} in nitrous oxide analgesia and in phenobarbital anesthesia, allowing a comparison with previous results on oxygen consumption, and (2) to test a recent proposal that 70% N_2O markedly reduces 1-CMR_{gl} in (mainly) cortical structures. Under 70% N_2O , 1-CMR_{gl} in frontal and parietal cortex was close to $0.7 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$. This value is in excellent agreement with previous values for "cortical" oxygen consumption. In phenobarbital anesthesia, 1-CMR_{gl} was lower than that expected from oxygen consumption, probably reflecting the fact that barbiturate anesthesia is accompanied by consumption of endogenous substrates. Experiments on adrenalectomized animals that were given local anesthesia and protected from external stimuli failed to demonstrate that 70% N_2O depresses 1-CMR_{gl} . In fact, N_2O was found to increase 1-CMR_{gl} in many structures. It is concluded that if 1-CMR_{gl} is lower in ventilated animals than in spontaneously breathing, conscious controls, the depression is more likely to be due to the neuromuscular blockade.

Key words: Brain metabolism, local glucose consumption, nitrous oxide analgesia, phenobarbital anesthesia, neuromuscular blockade

The development by Sokoloff et al. (1977) of the autoradiographic ^{14}C -deoxyglucose technique for measuring local cerebral glucose consumption in the brain (1-CMR_{gl}) has provided a new means of studying cerebral metabolic rates in physiological and pathological situations. By virtue of its resolution the method has the unique feature of allowing measurements of glucose consumption rates in discrete anatomical structures and functional units. In this way, novel metabolic information has been obtained on such diverse functions as those subserved by the visual, auditory and olfactory systems (for literature, see Sokoloff et al. 1977, Sokoloff 1977, 1978), on the effect of α - and β -adrenoceptor blockers (Savaki et al. 1978), as well as on local metabolic rates accompanying focal and generalized epileptic seizures (Collins et al. 1976, Siesjö & Abdul-Rahman 1979), or those following

spreading cortical depression (Shinohara et al. 1979) and cerebral ischemia (Levy & Duffy 1977).

In any study of cerebral metabolic rate it becomes a problem to define an appropriate control state. Ideally, such a state mimicks conditions prevailing in normal, resting subjects (or experimental animals). Many pathophysiological conditions, particularly those involving changes in lung ventilation, blood pressure, or body temperature, require artificial ventilation. Furthermore, immobilization is many times a prerequisite for appropriate sampling of tissue, cerebral venous blood, and CSF, or for control of physiological parameters. In immobilized animals, the choice of anesthetic or analgetic drugs is crucial. In their review article, Smith & Woolman (1972) conclude that, in man, 70% N_2O has small effects on CBF and only a mild depressant effect on CMR_{O_2} . Experiments on dogs

(Theye & Michenfelder 1968) and rats (Carlsson et al. 1976) have given similar results, indicating that 70% N₂O may provide an adequate control state in immobilized animals.

So far, extensive control data for 1-CMR_{gl} have been published only for unanesthetized and partially restrained rats and monkeys, and for rats subjected to superficial thiopental anesthesia (Sokoloff et al. 1977, Sokoloff 1978, Kennedy et al. 1978). Recently, Sakurada, Shinohara, Kennedy & Sokoloff (manuscript in preparation) compared 1-CMR_{gl} values obtained in spontaneously breathing rats, that were either partially restrained by a plaster cast or freely moving, to those measured in immobilized animals ventilated on 70% N₂O. Since their results indicate that 70% N₂O depresses 1-CMR_{gl} in cortical structures by up to 35% the authors question the assumption that values so obtained approximate those expected in the resting conscious state.

The present experiments were undertaken to critically assess 1-CMR_{gl} values obtained in ventilated rats maintained on 70% N₂O. The following questions were posed. (1) Are the 1-CMR_{gl} values measured in reasonable agreement with those expected from CMR_o values previously obtained in immobilized rats? In order to obtain data for this comparison, 1-CMR_{gl} was measured in animals maintained on 70% N₂O as well as in those given phenobarbital in a dose of 150 mg·kg⁻¹. These situations provide both "normal" and markedly reduced metabolic rates. Furthermore, since many experiments are conducted in fasted rats, 1-CMR_{gl} under 70% N₂O was assessed both in fed rats, and in those fasted for 24 h. (2) Does 70% N₂O depress glucose utilization in the brain? In order to answer this question 1-CMR_{gl} was measured in animals maintained either on 70% N₂O–30% O₂ or on 70% N₂–30% O₂. In all of these animals, pain and stressful reactions were minimized by adrenalectomy and local anesthesia.

METHODS

Operative techniques and experimental groups

The experiments were performed on male Wistar rats of a SPF strain, weighing 370–420 g. In the majority of the animals (see below), anesthesia was induced with 3% halothane. When unresponsive, the animals were tracheotomized and immobilized with an i.v. injection of tubocurarine chloride (0.5 mg·kg⁻¹). Ventilation was continued with a gas mixture of 70% N₂O and 0.7% halothane

in oxygen for the duration of the operation. The femoral arteries were cannulated, one for recording blood pressure and the other for obtaining blood samples for the determination of blood gases, pH, glucose concentrations and ¹⁴C-deoxyglucose activity. One femoral vein was cannulated to allow infusion of drugs and ¹⁴C-deoxyglucose. Body temperature, as measured in the rectum, was kept close to 37°C by external heating. Upon completion of the operative procedures the halothane supply was discontinued and heparin was given i.v. in a dose of 100 I.U. per animal.

The following 5 groups of animals were included in the study:

1. *Fasted*—70% N₂O. 6 animals were fasted for 18–20 hours prior to the experiment. Following the completion of operation they were maintained on 70% N₂O.

2. *Fed*—70% N₂O. In this and in the following groups, each comprising 4 animals, the animals had free access to pellet food until the day of operation. This group was in all other respects similar to group 1.

3. *Adrenalectomy—local anesthesia*—70% N₂O. In this group the adrenal glands were removed during the operation. Local anesthesia (about 1 ml of 1% xylocain® per animal) was infiltrated at suture sites. The animals had their ears plugged with cotton wool, they were blinded with a sterile ointment and then almost completely embedded in cotton wool (see Carlsson et al. 1977). Ventilation was then continued on 70% N₂O.

4. *Adrenalectomy—local anesthesia*—70% N₂. This group was identical to the previous one except for the fact that nitrous oxide supply was discontinued for 15 min before ¹⁴C-deoxyglucose injection.

5. *Phenobarbital* 150 mg·kg⁻¹. This group differed from the others in that anesthesia was induced with phenobarbital (150 mg·kg⁻¹ i.p.). However, the animals were ventilated with 70% N₂O and 0.7% halothane during operation, at the end of which ventilation was continued on 70% N₂ and 30% O₂.

After the completion of operative procedures all animals were allowed a steady state period of 30 min before the determination of 1-CMR_{gl} was begun (however, see group 4). During this time it was controlled that PaCO₂ was in the range 35–40 mmHg and that PaO₂ exceeded 100 mmHg.

Determination of 1-CMR_{gl}—theoretical background

The ¹⁴C-deoxyglucose technique is based on the following considerations. Deoxyglucose is transported from plasma to cerebral intracellular fluids by the same carrier mechanisms as is glucose and like glucose, it is phosphorylated by hexokinase. However, the further metabolism of deoxyglucose is impeded by the fact that it is a poor substrate for glucosephosphateisomerase (E.C.5.3.1.9) and for glucose-6-phosphate dehydrogenase (E.C.1.1.1.49). Since the deoxyglucose-6-phosphatase (E.C.3.1.3.9) activity is low in brain tissue the sugar is essentially trapped in the tissue as deoxyglucose-6-phosphate. The amount trapped at any given time after its introduction in the blood then equals the integral of the rate of phosphorylation by hexokinase during that interval of time.

A quantitative relationship thus exists between the

phosphorylation rate of glucose and the amount of deoxyglucose-6-phosphate formed which depends on the relative concentrations of the sugars and on their relative affinities for the carrier mechanisms and for hexokinase. Since the latter are known (see below) it is possible to determine glucose utilization rates at steady state (which equal the phosphorylation rates) by measuring the time course of the arterial plasma specific activities of the two sugars and the deoxyglucose activity in tissue. For calculations of glucose utilization rates with the equation described by Sokoloff et al. (1977) certain conditions should be fulfilled. (a) Plasma glucose concentrations should be relatively constant during the experiment. (b) The deoxyglucose should occur in tracer concentrations. (c) The extravascular tissue compartment should be homogeneous and all its parts should exchange directly with plasma. (d) Since measurements of ^{14}C -deoxyglucose activity in tissue are made by autoradiography it is not possible to distinguish between the free and phosphorylated forms. Hence, a sufficient period should pass after the injection of ^{14}C -deoxyglucose to allow most of the free form to disappear by phosphorylation or transport.

Calculation of 1-CMR_{gl} is made from the following equation

$$R_i = \frac{C_p^*(T) - k_1^* e^{-(k_2^* + k_3^*)T} \int_0^T C_p^* e^{(k_2^* + k_3^*)t} dt}{\left[\frac{\lambda \cdot V_m^* \cdot K_m^*}{\Phi \cdot V_m \cdot K_m} \right] \left[\int_0^T \left(\frac{C_p^*}{C_p} \right) dt - e^{-(k_2^* + k_3^*)T} \int_0^T \left(\frac{C_p^*}{C_p} \right) e^{(k_2^* + k_3^*)t} dt \right]}$$

R_i stands for glucose uptake rate per unit mass of tissue; C_p and C_p^* are the arterial plasma concentrations of glucose and deoxyglucose; T is the time of decapitation after tracer injection; $C_p^*(T)$ is the amount of radioactivity in the tissue of that time; k_1^* and k_2^* are the rate constants of carrier-mediated transport of deoxyglucose between plasma and tissue and between tissue and plasma, respectively, k_3^* is the rate constant of phosphorylation of DOG; λ is the ratio of the tissue distribution space for ^{14}C -deoxyglucose and glucose, Φ is the fraction of glucose that at steady state is further metabolized after phosphorylation, while K_m^* , V_m^* , K_m , V_m are the Michaelis-Menten constants and maximal phosphorylation rates for deoxyglucose and deoxyglucose, respectively.

In applying the equation, T was 45 min and the constants were those given by Sokoloff et al. (1977).

Determination of 1-CMR_{gl} procedures

50 μCi ^{14}C -deoxyglucose (337 $\text{mCi}\cdot\text{mMol}^{-1}$, New England Nuclear) in 0.5 ml of Krebs solution was infused over a period of 30 s with an infusion pump. 15 timed 100 μl samples (including a blank) were taken during the 45 min experimental period. The samples were centrifuged and the plasma was separated and frozen at -80°C for later determination of ^{14}C -activity and glucose concentration. After 45 min the animal was decapitated whereafter the brain was removed and frozen in isopentane chilled to -50°C .

Plasma glucose concentrations were determined fluorometrically with the hexokinase method (see Folbergrová et al. 1972). The ^{14}C -deoxyglucose activity was de-

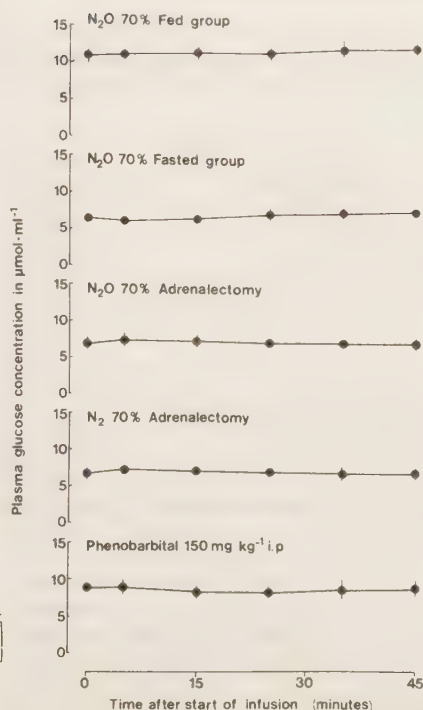


Fig. 1. The plasma glucose concentrations just before the start of deoxyglucose infusion and during the 45 min period of the expts. The values are means $\pm$ S.E.

termined by pipetting an aliquot of plasma into a scintillation vial containing 1 ml of a mixture of Soluene[®] and isopropanol (1:1, v/v). To the vials were then added 7.5 ml of a mixture of Instagel[®] and 0.1 N HCl (9:1, v/v). The samples were counted in a Nuclear Chicago liquid scintillation counter. The efficiency was calculated with the method of external standard ratio. The accuracy of this method was evaluated by adding an internal standard (^{14}C -hexadecane, Amersham) to the vials before recounting.

Determination of ^{14}C -activity in the tissue by autoradiography was done as described previously (Abdul-Rahman et al. 1979). When recalibrating the standards it was found that the values previously assigned to them were in error by about 5%. This was due to the fact that the volume of internal standard used was sufficient to induce some quenching. Accordingly, the CBF values given in that article (Abdul-Rahman et al. 1979) are about 5–10% too low.

RESULTS

The physiological parameters in the 5 experimental groups are given in Table 1. All animals had rectal

Table 1. Physiological parameters in experimental groups

The values are means $\pm$ S.E. MABP = mean arterial blood pressure

Experimental groups	Temp. (°C)	MABP (mmHg)	P _{O₂} (mmHg)	P _{CO₂} (mmHg)	pH
N ₂ O 70%, fed group (n=4)	37.0 $\pm$ 0.05	143 $\pm$ 5	115 $\pm$ 2	39.1 $\pm$ 0.4	7.37 $\pm$ 0.02
N ₂ O 70%, fasted group (n=6)	37.1 $\pm$ 0.1	135 $\pm$ 6	123 $\pm$ 2	36.8 $\pm$ 0.8	7.37 $\pm$ 0.01
N ₂ O 70%, adrenalectomy + local anesthesia (n=4)	37.1 $\pm$ 0.2	121 $\pm$ 7	104 $\pm$ 3	37.9 $\pm$ 1	7.41 $\pm$ 0.01
N ₂ O 70%, adrenalectomy + local anesthesia + N ₂ O (n=4)	37.3 $\pm$ 0.1	118 $\pm$ 6	110 $\pm$ 3	37.4 $\pm$ 0.8	7.44 $\pm$ 0.02
Phenobarbital, 150 mg $\cdot$ kg ⁻¹ (n=4)	37.4 $\pm$ 0.1	108 $\pm$ 5	117 $\pm$ 3	39.0 $\pm$ 1	7.40 $\pm$ 0.01

temperatures close to 37°C, Pa_{O₂} values close to or above 100 mmHg, Pa_{CO₂} values around 40 mmHg, and pH values close to 7.40. There was a tendency for blood pressure to decrease in the adrenalectomized animals ($P < 0.05$), and in those anesthetized with phenobarbital blood pressure was clearly decreased below "control" (70% N₂O, $P < 0.01$).

Fig. 1 shows the plasma glucose concentrations just before the start of deoxyglucose infusion and during the 45 min period of the expt. In all groups except that comprising the fed animals under 70% N₂O plasma glucose concentrations were in the range 5–8 μ mol $\cdot$ g⁻¹. As required (see Methods),

plasma glucose concentrations were stable throughout the period of the expt. with little variation between individual animals.

Local CMR_{gl} in nitrous oxide analgesia

Table 2 lists 1-CMR_{gl} values obtained in the groups of animals ventilated on 70% N₂O, and compares them to those measured by Sakurada et al. (in preparation) under similar conditions (70% N₂O and neuromuscular blockade). Local CMR_{gl} did not differ between fed and starved animals except in the cerebellar cortex which had a slightly higher rate in the starved group ($P < 0.05$). In view of the similarity in results between fed and starved rats the

Table 2. Local glucose consumption (1-CMR_{gl}) in animals ventilated on 70% N₂O with or without a prior fast of 18–20 h, as compared to data obtained by Sakurada et al. (in preparation) under similar conditions (see text)The values are means $\pm$ S.E.

Structures	Fasted n=6	Fed n=4	Combined n=10	Sakurada et al. n=5
<i>A. Superficial cerebral structures: cortex</i>				
Sensorimotor	0.68 $\pm$ 0.04	0.69 $\pm$ 0.04	0.69 $\pm$ 0.03	0.80 $\pm$ 0.03*
Auditory	0.92 $\pm$ 0.07	0.85 $\pm$ 0.04	0.89 $\pm$ 0.04	1.10 $\pm$ 0.06*
Visual	0.69 $\pm$ 0.03	0.60 $\pm$ 0.02	0.65 $\pm$ 0.03	0.76 $\pm$ 0.04*
Parietal	0.69 $\pm$ 0.04	0.69 $\pm$ 0.02	0.69 $\pm$ 0.02	0.79 $\pm$ 0.02**
Frontal	0.67 $\pm$ 0.03	0.76 $\pm$ 0.03	0.71 $\pm$ 0.03	0.87 $\pm$ 0.02**
<i>B. Deep cerebral structures</i>				
Hypothalamus	0.45 $\pm$ 0.03	0.37 $\pm$ 0.01	0.41 $\pm$ 0.02	0.43 $\pm$ 0.04
Caudate-putamen	0.86 $\pm$ 0.05	0.71 $\pm$ 0.03	0.80 $\pm$ 0.04	0.87 $\pm$ 0.06
<i>C. Mid-brain and Pons</i>				
Inferior Colliculus	1.00 $\pm$ 0.06	0.87 $\pm$ 0.04	0.95 $\pm$ 0.04	1.30 $\pm$ 0.11**
Pontine gray	0.55 $\pm$ 0.04	0.49 $\pm$ 0.03	0.52 $\pm$ 0.03	0.51 $\pm$ 0.05
<i>D. Cerebellum</i>				
Cerebellar cortex	0.55 $\pm$ 0.03	0.42 $\pm$ 0.01	0.50 $\pm$ 0.03	0.42 $\pm$ 0.03

* $P < 0.05$. ** $P < 0.01$.

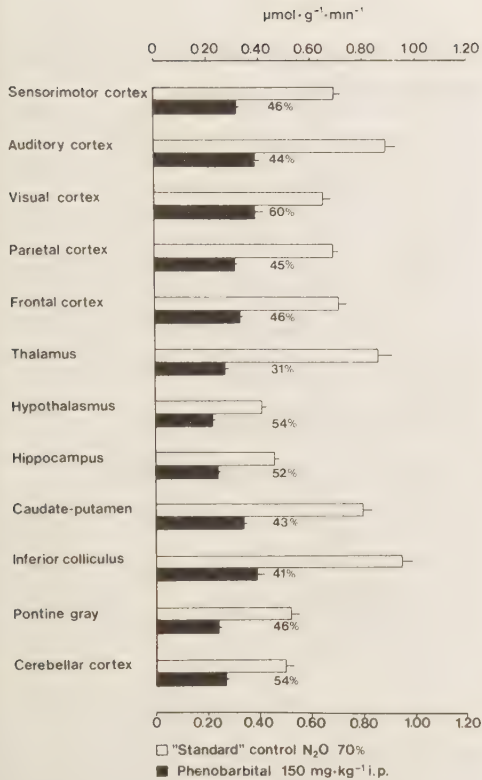


Fig. 2. The influence of phenobarbital anesthesia on local CMR_{gl} ($\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$) in the rat as compared with the standard 70% N_2O group. The numbers denote $1 - CMR_{gl}$ during phenobarbital anesthesia in percent of the standard control.

data were pooled for the comparison to results obtained by Sakurada et al. In areas with high metabolic rates (inferior colliculus and cerebral cortex) the present values were lower than those of Sakurada et al., the differences being about 30% in inferior colliculus and 10–20% in cortical areas. However, the relative proportion of $1 - CMR_{gl}$ between structures was very similar. In some other structures (particularly thalamus, hypothalamus, pontine gray and cerebellar cortex; to some extent also caudate-putamen) the values in the two series were in close agreement. In still others, a comparison could not be made since the densitometer used in the present study precluded measurements on very small structures.

Table 3. Comparison of local glucose consumption (CMR_{gl}) in frontal and parietal cortex and oxygen consumption (CMR_{O_2}) in animals ventilated on 70% N_2O or given 150 $\text{mg} \cdot \text{kg}^{-1}$ of phenobarbital

Anesthesia	CMR_{O_2} measured	CMR_{gl} calculated	CMR_{gl} found
70% N_2O	4.0 ^a	0.70	0.70
Phenobarbital 150 $\text{mg} \cdot \text{kg}^{-1}$	2.7 ^b	0.47	0.32

^a Berntman et al. (1979).

^b Nilsson & Siesjö (1975), with one aberrant value excluded (see discussion in article quoted).

2. Local CMR_{gl} in phenobarbital anesthesia

In view of the fact that the phenobarbital-anesthetized animals were paralyzed and artificially ventilated, it would seem most relevant to compare the values obtained to those of the "standard" group. Fig. 2 shows the absolute values obtained in the two groups and lists the percentage reduction in CMR_{gl} induced by the barbiturate in each structure studied. In most of the structures the reduction in $1 - CMR_{gl}$ was to about 50% of control, but there were exceptions. Thus, the reduction in CMR_{gl} was somewhat less pronounced in the visual cortex and somewhat more pronounced in inferior colliculus. In the thalamus, the reduction was excessive (to a third of control).

3. A comparison between glucose and oxygen consumption rates

Previously, CMR_{O_2} has been measured in animals maintained on 70% N_2O (e.g. Berntman et al. 1979) and in those given phenobarbital 150 $\text{mg} \cdot \text{kg}^{-1}$ (Nilsson & Siesjö 1975), experimental conditions being similar to the present ones. In these studies, we used a modification of the Kety & Schmidt (1948) technique, with sampling of cerebral venous blood from the caudal portion of the superior sagittal sinus. Assuming that this sampling site provides CMR_{O_2} values representative of mainly frontal and parietal cortex and that the CMR_{gl} values measured come close to the weighted averages for these structures we lumped together the values for these two cortical areas for comparisons. Furthermore, we assumed that 95% of the glucose consumed is oxidized, the remainder appearing as lactate (and pyruvate) in the venous effluent (see Hawkins et al. 1973, Norberg & Siesjö 1974). The resulting calcu-

Table 4. Comparison of 1-CMR_{gl} in animals that were adrenalectomized and given local anesthesia and ventilated on either 70% N_2O or 70% N_2

The values are means $\pm$ S.E.

Structures	70% N_2O	70% N_2
A. Superficial cerebral structures: cortex		
Sensorimotor	0.73 ± 0.04	0.64 ± 0.04
Auditory	0.97 ± 0.05	$0.72 \pm 0.03^{**}$
Visual	0.81 ± 0.05	$0.63 \pm 0.02^*$
Parietal	0.69 ± 0.02	0.63 ± 0.04
Frontal	0.81 ± 0.04	$0.64 \pm 0.04^*$
B. Deep cerebral structures		
Thalamus	1.05 ± 0.05	$0.67 \pm 0.03^{***}$
Hypothalamus	0.48 ± 0.02	0.41 ± 0.03
Hippocampus	0.73 ± 0.01	$0.55 \pm 0.05^{**}$
Caudate-putamen	1.07 ± 0.05	$0.75 \pm 0.04^{**}$
C. Mid-brain and Pons		
Inferior Colliculus	0.81 ± 0.06	0.66 ± 0.06
Pontine gray	0.55 ± 0.03	0.44 ± 0.04
D. Cerebellum		
Cerebellum cortex	0.52 ± 0.02	0.49 ± 0.04

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

lations (Table 3) showed an excellent agreement between expected and measured cortical CMR_{gl} in animals on 70% N_2O . However, in phenobarbital-anesthetized animals the autoradiographically measured CMR_{gl} values were lower than those expected from the CMR_{O_2} measurements (see Discussion).

4. The influence of nitrous oxide analgesia

As stated, a previous study showed no significant difference in CMR_{O_2} between animals ventilated on 70% N_2O and 70% N_2 (Carlsson et al. 1976). In these, stress, pain and discomfort were minimized by removal of the adrenal glands, by local anesthesia, and by measures instituted to reduce external stimuli. Using an identical preparation, we measured glucose consumption rates with and without 70% N_2O (Table 4). Unexpectedly, the results showed that 70% N_2O increased 1-CMR_{gl} in many of the structures studied. Thus, although there were no or only small differences in sensorimotor and parietal cortex, hypothalamus, inferior colliculus, pontine gray and cerebellar cortex, other structures (frontal, auditory and visual cortex, thalamus, hippocampus, and caudate-putamen) showed signifi-

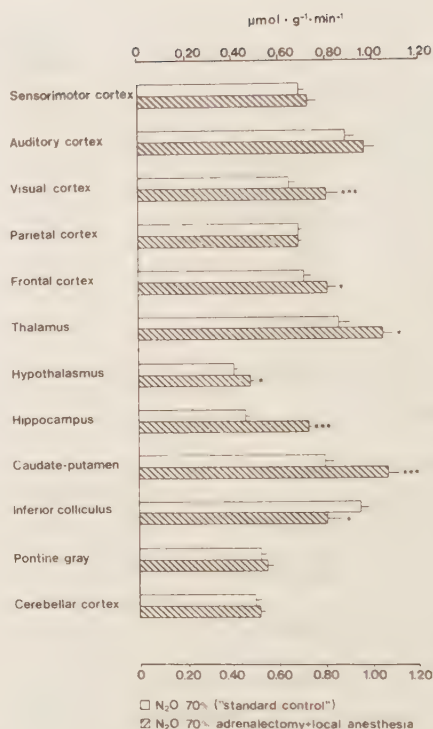


Fig. 3. Local CMR_{gl} in adrenalectomized and locally anesthetized animals ventilated on 70% N_2O (stippled bars) as compared to "standard" control animals maintained on 70% N_2O (open bars). The values are means $\pm$ S.E. in $\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

cantly increased CMR_{gl} with 70% N_2O . These results seem to exclude the possibility that 70% N_2O reduces metabolic rates in the brain (see Discussion).

Clearly, as Fig. 3 shows, a complex picture emerges when the results obtained on adrenalectomized nitrous oxide animals (Table 4) are compared to those obtained in the "standard" group (Table 2). Thus, in spite of the fact that both groups were artificially ventilated on 70% N_2O animals that were adrenalectomized, injected with a local anesthetic and protected against external stimuli had higher 1-CMR_{gl} values in frontal and visual cortex, thalamus, hippocampus and caudate-putamen, and a lower value in inferior colliculus. We conclude that one or more of these procedures influence local glucose consumption.

DISCUSSION

The present results are highly pertinent to the problem of obtaining proper control conditions for studies of physiological and pathophysiological events in the brain. When discussing the results it should be recalled, though, that such conditions may vary from one study to another. Clearly, for studies of many physiological events it must be desirable to compare results to those obtained in unanesthetized and spontaneously breathing animals. It would seem that, for these, the control states used by Sakurada et al. (in preparation, see also Sokoloff et al. 1977) are the appropriate ones. It has not been excluded, though, that values so obtained are influenced by the stress of partial restraint or of infusion and/or blood sampling in freely moving animals (see below).

As stated in the introduction, other studies require immobilization and some form of anesthesia/analgesia, e.g. that provided by 70% N₂O. The question has remained whether or not such measures markedly alter brain metabolism and blood flow. In the following, we will discuss the implication of the present results for these general problems.

1. Validity of $I\text{-CMR}_{\text{gl}}$ values in animals maintained on 70% N₂O

We recognize the difficulty of estimating a weighted average CMR_{gl} value for any given cerebral structure, and referring $I\text{-CMR}_{\text{gl}}$ and CMR_{O_2} values to a given, defined structure. However, since similar $I\text{-CMR}_{\text{gl}}$ values were obtained if repeated densitometry readings were taken from different parts of e.g. the frontal and parietal cortices (data not given), since values for frontal, parietal and sensorimotor cortex were similar, and since the superior sagittal sinus probably drains mainly cortical structures, we conclude that the present $I\text{-CMR}_{\text{gl}}$ values quantitatively correspond to previous measurements of CMR_{O_2} . Since the values are also close to those measured with a kinetic ¹⁴C-glucose technique (Borgström et al. 1976) there should be little doubt about the quantitative validity of the values obtained. True, in cerebral cortical structures, and in the inferior colliculus, our CMR_{gl} values were moderately but significantly lower than those measured by Sakurada, Shinohara, Kennedy & Sokoloff (in preparation). However, the differences were small enough to be explained by differ-

ences in metabolic characteristics between the rat strains used (Sprague-Dawley versus Wistar), or by unrecognized differences in experimental conditions. Such differences may explain why Sakurada et al. obtained local CBF values exceeding 2.2 ml·g⁻¹·min⁻¹ in all cortical areas except the visual cortex. To our knowledge, flow rates of this magnitude have not been recorded previously.

One further point should be emphasized. In view of the fact that about 20% of the brain oxygen consumption is accounted for by ketone body oxidation after a 24-hour fast (Ruderman et al. 1974) it may seem paradoxical that glucose consumption rates were similar in fed and fasted animals. However, since an increased proportion of glucose is metabolized to lactate (and pyruvate) in fasted animals and since arteriovenous differences in glucose concentration remain virtually unchanged (Hawkins et al. 1971, Ruderman et al. 1974) the present results are not surprising.

2. Validity of $I\text{CMR}_{\text{gl}}$ values in animals under phenobarbital anesthesia

The primary purpose of performing measurements on phenobarbital-anesthetized animals was to allow a comparison between CMR_{gl} and CMR_{O_2} . At first sight, it was surprising that a good correspondence was obtained in animals on 70% N₂O but not in those anesthetized with phenobarbital. In barbiturate-anesthetized animals, the calculated CMR_{gl} value (0.47 $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$), even if somewhat too high (see discussion of CBF technique by Berntman et al. 1979), clearly exceeds that measured with the autoradiographic technique in the appropriate cortical areas. We tentatively conclude that the difference is due to the fact that actual metabolic rate (i.e. oxygen consumption) exceeds glucose utilization. This can be explained by the fact that barbiturates retard glycolysis at the phosphofructokinase step, leading, at least initially, to mobilization of endogenous carbohydrate and amino acid substrates for oxidation (Chapman et al. 1978). If so, barbiturate anesthesia provides an example of conditions in which glucose and oxygen consumption rates are partly dissociated.

Previously, the influence of thiopental on $I\text{-CMR}_{\text{gl}}$ has been assessed (Sokoloff et al. 1977). Local CMR_{gl} was reduced by 40–50% in areas with high metabolic rate, particularly of cerebral cortical origin. Since thiopental was administered in doses that just extinguished the corneal reflex the values

may not be representative of deep barbiturate anesthesia. In the present material phenobarbital was given in doses that produced surgical anesthesia. As the results show, phenobarbital reduced CMR_{gl} in most structures to below 50% of control; in some, the reduction was to 35–40% of control. It should be emphasized that these values were obtained when a comparison was made to nitrous oxide controls. If "normal" control values are higher (see below) the relative depression of $1-CMR_{gl}$, induced by phenobarbital anesthesia, must be higher than indicated.

3. The influence of 70% N_2O

The results obtained on the two adrenalectomized groups of animals strongly argue against the proposition that 70% N_2O depresses cerebral metabolic rates. In parietal and frontal cortex differences in CMR_{gl} between animals maintained on 70% N_2O or 70% N_2 were either absent or small (see also data for sensorimotor cortex). Since samples drawn from the superior sagittal sinus probably contain blood from these areas the present results are in line with those showing that CMR_{O_2} does not differ between adrenalectomized animals ventilated with or without 70% N_2O (Carlsson et al. 1976). However, the present results unequivocally show that in many other cortical and subcortical structures 70% N_2O significantly enhances glucose consumption. This result may appear baffling. However, it has previously been shown that, in dogs, nitrous oxide enhances CMR_{O_2} (Theye & Michenfelder 1968). Possibly, increases in metabolic rates in some cerebral structures are related to the fact that nitrous oxide only induces state II anesthesia, a state which involves components of neurophysiological excitation (see Winters 1972).

4. Local CMR_{gl} values obtained in spontaneously breathing and in artificially ventilated animals

Values obtained from Sokoloff's group (Sokoloff et al. 1977, Sakurada et al., in preparation) suggest that $1-CMR_{gl}$, particularly in grey matter areas, is markedly reduced in animals ventilated on 70% N_2O with neuromuscular blockade. The present results do not corroborate the conclusion of Sakurada et al. that this reduction reflects a depressant effect of nitrous oxide on cerebral metabolism. There are two possible explanations for the difference in results obtained between spontaneously

breathing and artificially ventilated animals. First, it could reflect a reduction in cerebral metabolic rate due to the neuromuscular blockade, with an associated reduction in afferent stimuli. Second, it is possible, that partial restraint and experiments on freely moving animals induce a stress-related increase in $1-CMR_{gl}$ which is reduced when immobilized animals are ventilated on 70% N_2O . Evidence that some stress could be present has been published by Bühler et al. (1978) who found that gentle handling of rats for 30 s caused the plasma adrenaline concentration to rise to about 1000% of basal values.

At present, the cause of the differences in $1-CMR_{gl}$ values between spontaneously breathing and artificially ventilated animals is unresolved. Since these differences hamper quantitative conclusions regarding changes in $1-CMR_{gl}$ in a variety of physiological and pathophysiological situations, further work is warranted. It would seem that as long as experimental and control animals are treated similarly the differences discussed are less important. However, the present experiments emphasize that measures that have no known cerebral metabolic effects (adrenalectomy, local anesthesia, reduction of external stimuli) nevertheless alter $1-CMR_{gl}$ in many cerebral structures. Clearly, these results emphasize the necessity to adjust control conditions as closely as possible to the experimental situation.

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The effect of nitrous oxide on local cerebral
glucose utilization in rats.

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ABSTRACT

The influence of 70-80% N₂O on local CMR_{gl} in the rat brain was studied with the ¹⁴C-deoxyglucose method in minimally restrained, spontaneously breathing animals 75 min following discontinuation of halothane anaesthesia. Nitrous oxide was found to have but small effects on l-CMR_{gl} in the majority of the 25 structures analyzed. When corrections were made for a small difference in body temperature between N₂O-breathing animals and those breathing air, nitrous oxide was found to significantly increase l-CMR_{gl} in some subcortical structures by 15-25% (red nucleus, thalamus, the geniculate bodies and the superior colliculus), and to decrease l-CMR_{gl} in nucleus accumbens and sensorimotor cortex by comparable amounts. Thus, although nitrous oxide does not alter overall glucose utilization in the brain, it differentially affects CMR_{gl} in some brain structures.

INTRODUCTION

The effect of anaesthetics on cerebral blood flow and metabolic rate is of major concern to those who study experimental conditions requiring immobilization and some form of analgesia/anaesthesia. Since deep anaesthesia may profoundly affect the variables being studied many investigators have employed 70-80% N₂O as the only "anaesthetic", once the operative procedures have been completed. Thus, although nitrous oxide does not induce surgical anaesthesia, observations in cats suggest that its analgesic properties justifies its use in animals experiments (Venes et al. 1971).

There is at present no agreement on the effect of nitrous oxide on CBF, CMRO₂, and CMR_{gl}. Previous studies in man (reviewed by Smith and Wollman, 1972) suggested that 70% N₂O had little effect, if any, on CBF but gave no clear indication whether CMRO₂ was reduced or unchanged. However, a subsequent study showed that the combination of 70% N₂O and morphine sulfate (1 or 3 mg.kg⁻¹) neither altered CBF nor CMRO₂ or CMR_{gl} from awake, control values (Jobes et al. 1977).

In contrast, three studies in dogs have demonstrated that 70% N₂O increases CMRO₂ (and CBF). Using their venous outflow technique, Theye and Michenfelder (1968) noted an 11% increase in CMRO₂ in dogs that were given spinal anaesthesia and had their vagi cut. Similar techniques were used by Sakabe et al. (1978) who found that 60% N₂O, added to dogs maintained on 0.2% halothane, increased CMRO₂ by about

20%. With a background anaesthesia of 0.8% halothane, 60% N₂O did not consistently increase CMRO₂ and, in animals given thiamylal (8 mg.kg⁻¹), nitrous oxide did not increase CMRO₂ until the effects of the barbiturate had worn off (cf. similar effects of ketamine, as reported by Dawson et al. 1971). Since nitrous oxide has been reported to stimulate the sympathoadrenal system (Bahlman et al. 1971), Sakabe et al. (1978) studied the effect of reserpine administration (0.5 mg.kg⁻¹); however, this treatment did not curtail the increase in CMRO₂ (or CBF) following addition of 60% N₂O. In a subsequent study from the same laboratory the authors (Oshita et al. 1979) confirmed the results of Sakabe et al. (1978), using both the venous outflow technique and a modification of the Kety-Schmidt procedure.

Results obtained in rats (and mice) are less consistent. Working on ventilated rats that were adrenalectomized, given local anaesthesia, and protected from external stimuli, Carlsson et al. (1976) found no difference in CMRO₂ (or CBF) between animals ventilated on 70% N₂O or 70% N₂. The modified Kety-Schmidt technique used by these authors probably measures CMRO₂ (and CBF) in mainly frontal, parietal, and sensorimotor cortex. On the other hand, Gibson and Duffy (1981), measuring arteriovenous differences in oxygen content (tail artery versus confluent sinus) and supratentorial CBF (by a fractional extraction technique) in unparalyzed but mechanically restrained rats, found that 70% N₂O increased CMRO₂ by 33%. Available autoradiographic data suggest that such inconsistencies in results may reflect regional differences in nitrous oxide effects. Thus measurements of CMRgl in ventilated rats showed no effect of N₂O in frontal, parietal and sensorimotor cortex, but increases in CMRgl in other structures, including auditory cortex, thalamus, caudoputamen, and hippocampus (Ingvar et al 1980).

Other studies, in which different techniques were used to estimate cerebral metabolic rate, do not clarify the metabolic effects of nitrous oxide. Thus, whereas one group of workers, using the "closed system method" of Lowry et al. (1964) in mice, concluded that 70% N₂O does not measurably effect cerebral metabolic rate (Ratcheson et al. 1977), another group recently concluded that nitrous oxide induces an increased cerebral energy demand (Rosenthal et al. 1981). In that study, though, metabolic rate was estimated from the ratio of

reduction/oxidation of cytochrome a-a₃ in rats with a cerveau isolé lesion.

Clearly, there is a need to assess the influence of nitrous oxide on cerebral metabolic rate and to separate any effect present from that due to immobilization and artificial ventilation. We recently reported that 70% N₂O, administered to awake, minimally restrained rats, had but small effects on local CBF, as measured autoradiographically in twentytwo brain structures (Dahlgren et al. 1981). Thus nitrous oxide failed to induce significant increases (at the $p < 0.01$ level) in any of the structures measured, and reduced CBF was found in inferior colliculus, inferior olive and the hypothalamus. In the present study, we evaluated the effect of 70% N₂O on local CMRgl, using the same experimental preparation.

MATERIALS AND METHODS

Chemicals

Nitrous oxide (Alfax, Malmö) was mixed with oxygen in the proportion 70:30 (v/v). Heparin (Vitrum, Stockholm) was diluted with Krebs-Hensleit solution to yield an activity of 300 IU.ml⁻¹. ¹⁴C-deoxyglucose (337 mCi.mmol⁻¹, New England Nuclear) was supplied in a 10% ethanol solution. Before each experiment, an aliquot of this solution was evaporated in a stream of nitrogen gas, and the substance was dissolved in Krebs-Hensleit solution to yield an activity of 100 μ Ci .ml⁻¹. ¹⁴C-hexadecane standard was obtained from the Radiochemical Center (Amersham, England).

Animals

The experiments were performed on male Wistar rats of a SPF strain (Møllegaards Avlslaboratorium, Copenhagen), weighing 300-370g. The animals were allowed free access to tap water. The supply of pellets (Astra-Ewos, Södertälje) was not withdrawn until the morning of the day of the experiments. As in the previous study (Dahlgren et al. 1981) measurements were performed in the late afternoon to reduce ambient noise.

Operative and Sampling Techniques

For measurements of 1-CMRgl 75 min following discontinuation of halothane anaesthesia the operative procedures and the handling of the

animals were as previously described (Dahlgren et al. 1981). In summary, the animals were anaesthetized with 3-4% halothane in $N_2O:O_2$ (70:30) and maintained on 1% halothane in $N_2O:O_2$, delivered on a face mask, for insertion of tail arterial and venous catheters, as well as of a thermistor probe in the colon descendens. After the completion of these preparations, the animals were heparinized, and the face mask was removed. The animals were then allowed to recover from the anaesthesia in a specially designed Perspex cage which prevented the animals from turning, but allowed manipulations with the tail catheters.

After a recovery period of 30 min, the animals were either allowed to breathe room air or were given a gas mixture of 70-80% N_2O in O_2 for 45 min before the infusion of ^{14}C -deoxyglucose was started. Prior to this infusion, and during the 45 min infusion period, repeated arterial blood samples were taken for analyses of PO_2 , PCO_2 , pH, and glucose concentration with frequent control of blood pressure. We attempted to maintain body temperature to 37-38°C by intermittent heating of the cage with a lamp. However, it was found that air-breathing animals maintained a body temperature of 38°C even without heating, while those breathing the $N_2O:O_2$ gas mixture had body temperatures of less than 37.5 even when the lamp was placed close to the cage. Since the original groups studied ($n=6$ in each) differed in body temperature by 0.8°C, four additional N_2O -breathing animals were added at later occasion. In these, body temperature was maintained close to 38°C by removing the aluminum foil from the back of the perspex cage, thus allowing a more intense heating of the animals with the heating lamp..

At the end of the infusion period the animals were decapitated and the brains processed for quantitative autoradiography (see below).

Methods and Analytical Procedures

We measured arterial PO_2 , PCO_2 , and pH immediately after sampling, using microelectrodes (Eschweiler and Co, Kiel, and Radiometer, Copenhagen), with due corrections for any deviation of body temperature from 37°C. Plasma glucose concentrations were measured on a Beckman Glucose Analyzer 2. The instrument was calibrated with a series of glucose standards; besides, the accuracy of the determina-

tions was ascertained by comparisons with a fluorometric hexokinase method.

Local CMR_{gl} was measured according to Sokoloff et al. (1977), with details of procedures as given in a previous communication (Ingvar et al 1980). In brief, a 0.5 ml solution containing 50 uCi of deoxyglucose was infused over 30 sec with an infusion pump. At least 14 timed blood samples (including a pre-infusion blank) of 100 ul each were taken during the 45 min experimental period. The samples were immediately centrifuged and the plasma samples frozen at $-80^{\circ}C$ for later determination of ^{14}C -activity and glucose concentrations. At the end of the experimental period the animal was decapitated whereafter the brain was removed and frozen in isopentane chilled to $-50^{\circ}C$.

We determined plasma ^{14}C -activities by liquid scintillation counting, and checked the counting efficiency by adding an internal standard (^{14}C -hexadecane). For determining tissue ^{14}C -activities, the brain was sectioned at $-20^{\circ}C$ with a Leitz sledge microtome. The 20 μ m thick sections were applied to x-ray film together with a set of calibrated ^{14}C -standards. The films were exposed for 7 days. We evaluated the density of 25 different structures by a densitometer having an aperture of 1 mm. For calculations of $l-CMR_{gl}$ we used the equation described by Sokoloff et al. (1977) and the kinetic constants given by these authors for normal rats.

Statistics

Statistical differences were calculated by the unpaired student t -test. Since comparisons between control and experimental groups were made for 25 structures, the differences regarded as significant had a $p < 0.01$.

RESULTS

Physiological parameters in the three groups are given in Table 1. PCO_2 and pH values obtained in the air-breathing and N_2O/O_2 -breathing groups were virtually identical to those previously described for animals treated similarly (Dahlgren et al. 1981). Administration of the N_2O/O_2 mixture slightly elevated arterial PO_2 . Plasma glucose concentrations were similar in the three groups. In the original group of N_2O -breathing animals ($n=6$) body temperature was 36.9, 37.0, 37.0, 37.0, 37.2, and 37.8 $^{\circ}C$, respectively, while the additional four

TABLE 1. Physiological parameters in air-breathing and in N₂O/O₂-breathing animals.

Experimental groups	Temp C	MABP mmHg	PCO ₂ mmHg	PO ₂ mmHg	pH	Glucose umol/ml
Air (n=6)	38.0±0.1	108±4	40.5±0.7	78±2	7.42±0.01	7.7±0.2
N ₂ O/O ₂ (n=10)	37.5±0.2	111±2	38.8±0.8	105±2	7.40±0.01	9.5±0.4

Values are given as mean±S.E.

Significant differences ($P < .05$) were noted between values for PO₂ and glucose. MABP= mean arterial blood pressure

animals had values of 37.9, 38.1, 38.2, and 38.2 °C, respectively. In the combined group the body temperature was 0.5°C less than in the air breathing group ($p < 0.07$). This temperature difference will be further discussed below.

All values in Table 1 were those measured prior to decapitation of the animals. However, physiological variables showed little variation during the 45 min sampling period. Plasma glucose concentrations also remained stable during that period. Only one animal showed signs of excitation, as evidenced by forceful movements, a reduction in PCO₂, and an increase in blood glucose concentration (cf. Dahlgren et al. 1981). This animal was excluded from the material.

The effects of 70% N₂O on l-CMRgl are shown in Table 2. The areas analysed were arbitrarily divided into "motor", "sensory" and "limbic" structures, and cerebellum. As the results demonstrate, interanimal variations were small. The data reveal that administration of the N₂O:O₂ mixture had but small effects on l-CMRgl. Thus nitrous oxide was found to significantly increase l-CMRgl in the red nucleus and the lateral geniculate body. In the medial geniculate body the difference was almost significant ($p = .014$). Nitrous oxide failed to reduce l-CMRgl in any of the structures analyzed, but a suggested reduction occurred in the inferior colliculus ($p = .012$).

Since the last four of the N₂O-breathing animals (body temperature of 38°C) were done at a later occasion and since the results were somewhat more variable than in the original group, we corrected the values obtained in the original group to 38°C for comparisons with the air breathing group. In each animal the l-CMRgl values for each structure were corrected by using temperature factors derived from

Table 2. Local cerebral glucose utilization in air-breathing and N2O/O2-breathing rats.

	Air n=6	N2O/O2 n=10
Frontal cortex	0.67 + 0.01	0.66 + 0.03
Caudoputamen	0.70 + 0.01	0.71 + 0.03
Globus pallidus	0.33 + 0.02	0.35 + 0.02
Substantia nigra	0.42 + 0.01	0.43 + 0.02
Red nucleus	0.50 + 0.01	0.57 + 0.01 *
Nucleus accumbens	0.62 + 0.02	0.53 + 0.03
Cerebellum	0.39 + 0.01	0.39 + 0.01
Sensorimotor cortex	0.72 + 0.01	0.64 + 0.03
Parietal cortex	0.68 + 0.02	0.65 + 0.03
Thalamus	0.66 + 0.02	0.82 + 0.05
Lateral thalamus	0.63 + 0.01	0.69 + 0.03
Visual cortex	0.69 + 0.04	0.66 + 0.03
Lateral geniculate body	0.55 + 0.03	0.70 + 0.03 *
Superior colliculus	0.56 + 0.01	0.64 + 0.03
Auditory cortex	0.82 + 0.03	0.91 + 0.04
Medial geniculate body	0.68 + 0.03	0.80 + 0.03
Inferior colliculus	1.11 + 0.05	0.94 + 0.03
Temporal cortex	0.46 + 0.02	0.44 + 0.03
Cingulate cortex	0.63 + 0.02	0.64 + 0.03
Hippocampus	0.48 + 0.02	0.46 + 0.02
Amygdala	0.43 + 0.02	0.43 + 0.03
Septal nucleus	0.36 + 0.02	0.35 + 0.02
Habenula	0.67 + 0.01	0.66 + 0.03
Mamillary body	0.57 + 0.02	0.58 + 0.05
Hypothalamus	0.37 + 0.02	0.38 + 0.02

Values are given as Mean + S.E. in Umol/g/min

* denotes significant difference ($p < 0.01$)

McCulloch et al (1982), assuming an intrastructural linearity in temperature induced changes in l-CMRgl, between normothermic and hypothermic animals. A comparison of control and temperature corrected nitrous oxide group revealed the following (fig 1). First nitrous oxide significantly increased l-CMRgl in the red nucleus (+16%), thalamus (+18%), lateral geniculate (+31%), superior colliculus (+18%), and probably also in the medial geniculate ($p=0.012$). Significant reductions in l-CMRgl were found in nucleus

accumbens (-16%) and sensorimotor cortex (-16%). Overall, though, the changes were small.

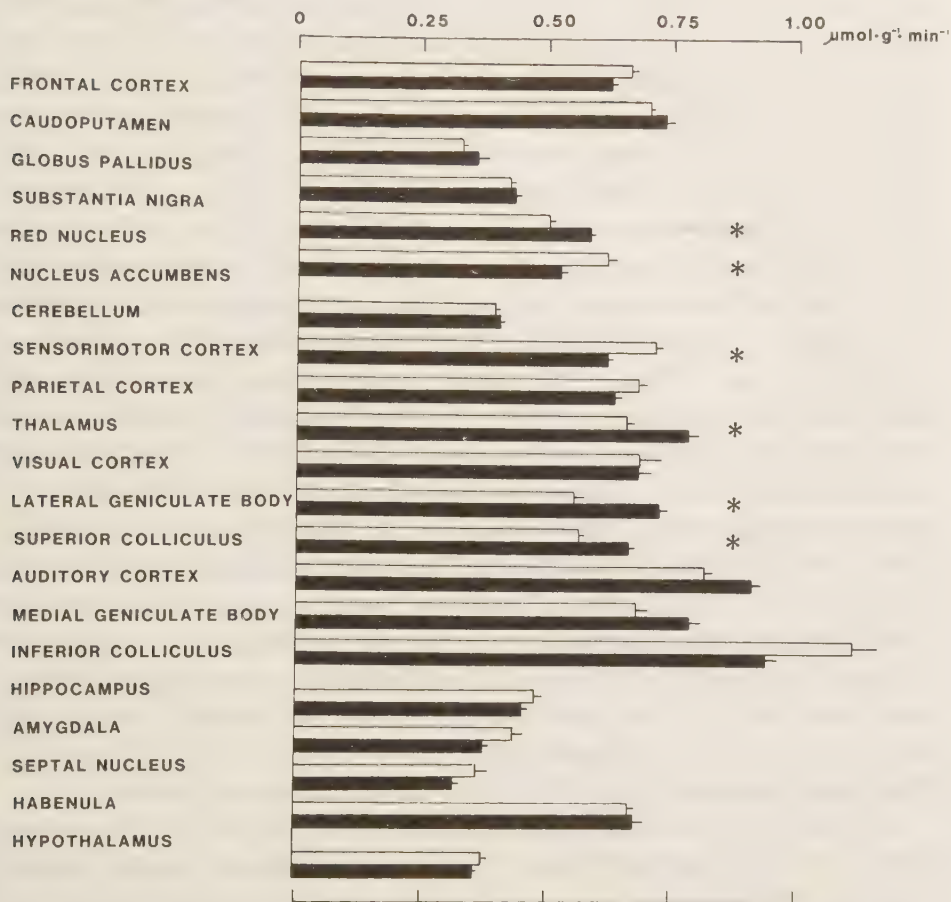


Fig 1. Local cerebral glucose utilization in airbreathing (open bars) and N₂O/O₂ breathing rats (black bars). The values are corrected for a temperature difference between groups (see text) . * p < .01

DISCUSSION

As discussed in the preceeding article (Dahlgren et al 1981) we have reasons to believe that the procedures used for studying awake rats yields values that are minimally influenced by excitation or stress, and that the model lends itself for evaluation of effects of

nitrous oxide (and other drugs). Admittedly, the recovery period following discontinuation of halothane anaesthesia is so short that it is difficult to exclude a lingering effect of the anaesthetic. However, unpublished results demonstrate that when air breathing animals were allowed 6 hrs of recovery from the halothane anaesthesia a significant change only occurred in the auditory cortex (+30%). Furthermore, the present comparison seems valid since both air breathing and N₂O-breathing animals had similar recovery periods, following discontinuation of halothane anaesthesia.

When the present results are considered, it should be recalled that nitrous oxide alone only induces stage 2 anaesthesia, a stage accompanied by signs of excitation and by cataleptoid behaviour (Winters et al 1972). One cannot expect, therefore, that the metabolic (or circulatory) effects of nitrous oxide should resemble those induced by general anesthetics of the barbiturate type, or that any effects elicited should involve changes of a uniform type (cf barbiturate data reported by Sokoloff et al 1977, Ingvar et al 1980).

The present results demonstrate that 70% N₂O has but small effects on CMRgl in the rat brain. Thus, in more than two thirds of the structures analysed l-CMRgl in N₂O-breathing animals were within 15% of control and when significant alterations from control were induced, these involved both increases and decreases. The present results are, therefore, in close agreement with those reported for man (Jobes et al 1979), rat (Carlsson et al 1977), and mouse (Ratcheson et al 1977).

In drawing conclusions regarding effects of nitrous oxide on local CMRgl values we have confidence in the values obtained in the original group of six N₂O-breathing animals after temperature correction. This is due to the fact that the experiments were performed at the same time as the controls and that the interanimal variability was small. Since temperature factors have been determined for rat cerebral structures (McCulloch et al 1982), these were used for correcting the values to a common temperature of 38°C. It does not seem established that the regional temperature coefficients reported by McCulloch et al (1982) differed significantly from the mean of 5-6% change in CMRgl per degree centigrade in change in temperature, a value virtually identical to that previously reported in rat cerebral cortex (Hägerdal et al 1975, see also Michenfelder and Theye 1968). However, conclusions drawn from the comparison were not different whether

individual factors (2-8%) or a common factor of 5% was used. Accepting the data of fig 1 as accurate we must conclude that nitrous oxide, although not altering overall cerebral metabolic rate, leads to a "redistribution" of local metabolic rate within the brain. Thus, increases (15-25%) in CMRgl were noted in four subcortical grey structures (red nucleus, thalamus, lateral geniculate, and superior colliculus), with a suggested increase in a fifth (medial geniculate). Equally large decreases were noted in nucleus accumbens and sensorimotor cortex. Although we consider these regional changes unequivocal, we can only speculate on their functional basis.

The present results are obviously at variance with those reported in dogs (Theye and Michenfelder 1968, Sakabe et al 1978, Oshita et al 1979) and those obtained in unparalyzed but restrained rats (Gibson and Duffy 1981). All experiments on dogs were carried out on paralyzed and artificially ventilated animals and, as judged by the marked decrease in arterial PCO₂ in the rats studied by Gibson and Duffy (1981), their animals were subjected to stress. A possible explanation for the difference in results may be that the effects of nitrous oxide are different during immobilization and in stressful situations. Whatever is the explanation, the present results demonstrate that 70% nitrous oxide does not lead to a uniform increase in metabolic rate.

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Local cerebral blood flow in the brain during bicuculline-induced seizures and the modulating influence of inhibition of prostaglandin synthesis

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The purpose of this study was to measure changes in local cerebral blood flow (l-CBF) during generalized seizures, and to study whether or not formation of prostaglandins or related substances contributes to the increased flow rates. Seizures were induced in ventilated rats maintained on 70% N₂O and 30% O₂ by the i.v. injection of the GABA receptor blocker bicuculline (1.2 mg · kg⁻¹). Formation of prostaglandins was inhibited by the administration of the fatty acid cyclo-oxygenase inhibitor indomethacin (10 mg · kg⁻¹). Local CBF in 21 defined brain structures was measured autoradiographically with ¹⁴C-iodoantipyrine as the diffusible tracer. After 20 min of continuous seizure activity l-CBF increased 1.5–5-fold, the smallest increases (<200% of control) being observed in frontal and auditory cortex and in the caudoputamen, and the largest (>400% of control) in substantia nigra, thalamus, visual cortex, lateral geniculate and hypothalamus. In general, the largest increases in l-CBF occurred in sensory and limbic systems (and hypothalamus) while motor systems showed a pronounced variability. In the majority of structures examined indomethacin failed to modify the CBF response during seizures. Although this result suggests that seizures, in contrast to hypercapnia, lead to an increased CBF by other mechanisms than those related to prostaglandin formation, some structures (nucleus ruber, cerebellum, and superior colliculus) showed a clearly reduced l-CBF in indomethacin-treated animals.

Epileptic seizures of whatever cause are accompanied by an increased cerebral oxygen consumption (CMR_{O₂}) and cerebral blood flow (CBF) (for literature see Plum & Duffy 1975, Meldrum & Nilsson 1976). Although the increase in CBF seems secondary to the enhanced metabolic rate the coupling mechanisms remain undefined. Several studies indicate that the cerebral vessels dilate in response to products of an altered cerebral metabolism, or, more specifically, to increases in extracellular concentrations of H⁺ and adenosine (Howse et al. 1974, Rubio & Berne 1976, Kuschinsky & Wahl 1979). However, measurements during bicuculline-induced seizures in rats have shown that CBF may increase dramatically before extracellular pH falls and in the absence of a measurable elevation of tissue adenosine concentration (Caspers &

Speckmann 1972, Nilsson et al. 1978, Rehncrona et al. 1979).

Recent results have demonstrated that indomethacin, a potent inhibitor of the fatty acid cyclo-oxygenase in brain tissue (Flower & Vane 1972, Wolfe et al. 1976, Abdel-Halim et al. 1978), markedly reduces CBF at normal CO₂ tensions and dramatically curtails the cerebrovascular response to hypercapnia (Pickard & MacKenzie 1973, Sakabe & Siesjö 1979, see also Discussion) but has no influence on the cerebral circulatory response to hypoxia (Sakabe & Siesjö 1979). These results indicate that some undefined prostaglandins or related substances help to maintain normal cerebrovascular tone and to mediate the response to hypercapnia but that they are not generally responsible for cerebrovascular dilatation.

At present, information on regional changes in CBF during generalized seizures is restricted. Measurements of regional CBF with a ^{14}C -antipyrine technique in cats during pentylenetetrazole seizures have been reported (Lorenzo et al. 1972). Interpretation of such data is hampered by the fact that the isotope grossly underestimates CBF, especially at high flow rates (Eklöf et al. 1974, Eckman et al. 1975). Recently two groups of workers have measured regional CBF during experimentally induced seizures. Mueller et al. (1979) induced seizures in cats by bicuculline and pentylenetetrazole and estimated CBF by a microsphere technique in a limited number of structures. The results showed a more marked increase in CBF in thalamus-midbrain and cerebrum than in the medulla and cerebellum. However, the method precluded a resolution of CBF changes in more discrete brain structures. Horton et al. (1980) induced seizures by bicuculline injection in rats and measured regional CBF by using both a microsphere and a ^{14}C -iodoantipyrine technique. To obtain regional values 13 brain structures were dissected for counting of radioactivity.

The objective of the present study was two-fold. First, we wanted to analyse the increase in CBF during seizures in all those structures that can be visualized by quantitative autoradiography with ^{14}C -iodoantipyrine as the diffusible tracer. Second, we wished to study whether or not prostaglandin-like substances influenced CBF during seizures (see Discussion). To that end one group of animals was given indomethacin prior to induction of seizures.

MATERIALS AND METHODS

Materials

Bicuculline methochloride (Sigma Chemical Co.) was dissolved in 0.1 N HCl and brought to pH 5–6 with 0.1 N NaOH to a concentration of $1.2 \text{ mg} \cdot \text{ml}^{-1}$. *Phentolamine* was prepared from Regitin®, Ciba, by dilution with physiological NaCl to yield a solution of $1 \text{ mg} \cdot \text{ml}^{-1}$. *Indomethacin* (Conforid®, Dumex) was prepared in sterile water solution containing $5\text{--}10 \text{ mg} \cdot \text{ml}^{-1}$. ^{14}C -iodoantipyrine (Amersham) with a specific activity of $54 \text{ mCi} \cdot \text{mmol}^{-1}$ was delivered in ethanol-solution. Before each experiment the ethanol was evaporated and the ^{14}C -iodoantipyrine was dissolved in $0.6\text{--}0.7 \text{ ml}$ Krebs solution.

Operative and sampling techniques

Male Wistar rats, weighing $255\text{--}390 \text{ g}$, had free access to pellet food (San-Bolagen, Malmö) and tap water until the time of operation. The animals were anesthetized in a jar with a gas mixture containing 3% halothane and 65%

nitrous oxide in oxygen. When unresponsive they were tracheotomized, connected to a Starling type respirator (Braun, Melsungen), paralyzed with tubocurarine chloride ($0.5 \text{ mg} \cdot \text{kg}^{-1} \text{ i.v.}$) and ventilated on 1% halothane and 70% N_2O in oxygen. Body temperature, as measured in the rectum, was kept close to 37°C by means of a heating bulb.

One femoral artery was cannulated for blood pressure recording and sampling of blood for analyses of pH, blood gases and glucose concentration. One brachial catheter was inserted to obtain arterial samples for measurements of local CBF (I-CBF, see below). This catheter was inserted $5\text{--}10 \text{ mm}$ into the vessel and cut $10\text{--}15 \text{ mm}$ outside the vessel to avoid distortion of the ^{14}C -iodoantipyrine curve due to "smearing". Both femoral veins were cannulated to allow infusion of ^{14}C -iodoantipyrine and of drugs (see below). Needles were inserted into the scalp to record the EEG by bipolar leads on an Elema machine (Elema, Stockholm). Upon termination of the operative procedure the halothane supply was discontinued and the animals were ventilated on 70% N_2O and 30% O_2 for at least 30 min before the experiment was started.

Measurements of local CBF

Local CBF was measured with the technique described by Landau et al. (1955) as modified by Sakurada et al. (1978). In brief, the method involves a constant i.v. infusion of ^{14}C -iodoantipyrine with repeated sampling of arterial blood and decapitation of the animal. CBF is then calculated from the integrated arterial curve and from the tissue concentration of ^{14}C -iodoantipyrine, the latter being determined with quantitative autoradiography. Details of procedure have been given in a preceding communication (Abdul-Rahman et al. 1979). In summary, $30\text{--}50 \mu\text{Ci}$ of ^{14}C -iodoantipyrine dispensed in 0.4 ml of Krebs-Henseleit solution was infused at constant rate for 30 s. Blood samples were obtained from the catheter in the brachial artery just before and every third s during the infusion. At 30 s the animal was decapitated; the brain was then removed from the skull (within 3 min) and immediately immersed in a cup containing isopentane chilled to -55 to -70°C . The ^{14}C activity per unit volume blood was measured in a β scintillation counter. The ^{14}C activity in brain tissues (per unit mass) was evaluated by autoradiography. Slices of brain ($20 \mu\text{m}$) were cut in a Leitz sledge microtome at -20°C and applied to an X-ray film which was then exposed for one week. A series of calibrated ^{14}C -standards was applied to each film together with the brains. The film was then evaluated manually with a densitometer.

The procedures used in this study differed from those previously described (Abdul-Rahman et al. 1979) in two respects. First, a minor error in the calibration of the standards was corrected (see Ingvar et al. 1980). Second, arterial samples were taken from the brachial rather than the femoral artery. Our experience has shown that sampling from the femoral artery may slightly underestimate the rate of increase in ^{14}C -activity in blood probably because the tip of the femoral artery catheter is not always exposed to rapidly circulating blood. Thus, the present technique should give a more accurate estimate of CBF at high flow rates.

Local CBF was calculated as described by Landau et al.

Table 1. *Physiological parameters in the experimental groups*

The values are means $\pm$ S.E. MABP = mean arterial blood pressure

Experimental groups	MABP (mmHg)	P _{CO₂} (mmHg)	Temp. (°C)
Bicuculline (n=4)	119 $\pm$ 4	37.2 $\pm$ 1	37.3 $\pm$ 0.2
Bicuculline + indomethacin (n=4)	118 $\pm$ 3	37.0 $\pm$ 2	37.3 $\pm$ 0.3

(1955) (see also Freygang & Sokoloff 1958). In spite of the sampling modification made it was not possible to accurately determine I-CBF in some structures at the excessively high flow rates encountered. The I-CBF equation gives a logarithmic relationship between the ^{14}C -activity in a structure and flow rate. Our experience shows that repeated densitometry readings gives a standard deviation of about 2%. In some structures the ^{14}C activity becomes so high that one reaches the steep slope of the (logarithmic) flow curve. We chose to consider values inaccurate when a 2% error in the determination of ^{14}C activity corresponded to a 10% variation in I-CBF, as evaluated in each individual animal. If activities were higher I-CBF was assigned a value determined at the "breakpoint". This means the I-CBF in some structures were underestimated, as indicated in Table 2 (see below).

Experimental groups

Local CBF was evaluated in two groups, each comprising four animals. In one group, the animals were injected intravenously with indomethacin 30 min before seizures were induced. In the other group, no injection was made. In all animals seizures were induced by the i.v. injection of bicuculline (1.2 mg $\cdot$ kg $^{-1}$).

The onset of seizures induced with bicuculline (or pentylentetrazole) is accompanied by a massive sympathetic discharge and by a pronounced increase in blood pressure that disrupts the blood-brain barrier and leads to extravasation of proteins (Lorenzo et al. 1972, Johansson & Nilsson 1977, Mueller et al. 1979). In order to avoid these events, the following procedures were adopted (cf. Meldrum & Nilsson 1976). Before bicuculline was injected blood was withdrawn from the artery until blood pressure was close to 120 mmHg. A slow injection of phentolamine was then given. When blood pressure started to fall, bicuculline and phentolamine was injected simultaneously (into the two femoral veins). By trial and error the phentolamine dose was chosen that limited the ictal blood pressure peak to 20–40 mmHg. No further phentolamine was given. After a variable time (5–15 min) blood pressure fell spontaneously. Reinfusion of shed blood was then given so as to maintain mean pressure at about 120 mmHg. In two animals (one in each group) the seizure discharges were attenuated (after 14 and 17 min). An additional injection of bicuculline (0.3 mg $\cdot$ kg $^{-1}$) was then given. Results obtained in these animals did not differ from those of the others.

After 20 min of (continuous) seizure activity ^{14}C -iodoantipyrine was infused for I-CBF estimation. Values obtained were compared to control values determined in a parallel series of experiments (Dahlgren, N., Nilsson, B., Sakabe, T., Siesjö, B. K. in preparation). These values were obtained with techniques that in every essential detail were identical to those employed in the present study.

Statistics

Differences between groups with respect to physiological parameters were statistically evaluated with the Student's *t*-test. Since the I-CBF could not be assumed to be normally distributed Mann-Whitney U-test was applied. Means $\pm$ S.E. were calculated in the conventional way.

RESULTS

In both groups, i.e. in uninjected and indomethacin-injected animals, the pattern of seizure discharge was identical to that previously reported (Chapman et al. 1977). Thus, indomethacin did not measurably modify the seizures provoked by bicuculline. In all animals except one, peak mean arterial blood pressure was below 160 mmHg. In one animal, pressure

Table 2. *Local cerebral blood flow (I-CBF) in bicuculline-injected animals with and without prior administration of indomethacin 10 mg $\cdot$ kg $^{-1}$*

The values (ml $\cdot$ g $^{-1}$ $\cdot$ min $^{-1}$) are means $\pm$ S.E. The figures appearing after the experimental groups represent the number of results assigned "break point" of the individual animal (see Methods)

	Bicuculline (n=4)	Bicuculline + indomethacin (n=4)
1. Frontal cortex	2.63 $\pm$ 0.66	3.52 $\pm$ 0.31
Nucleus ruber	5.26 $\pm$ 0.72 1	2.28 $\pm$ 0.22
Caudatus putamen	2.19 $\pm$ 0.42	1.87 $\pm$ 0.14
Substantia nigra	4.96 $\pm$ 0.49	5.48 $\pm$ 0.30
Cerebellum	2.81 $\pm$ 1.08	1.13 $\pm$ 0.10
2. Sensory motor cortex	5.49 $\pm$ 0.51	6.92 $\pm$ 0.25 3
Parietal cortex	4.69 $\pm$ 0.34	5.91 $\pm$ 0.16
Thalamus	6.25 $\pm$ 0.66 3	7.05 $\pm$ 0.16 4
Lateral thalamus	6.11 $\pm$ 0.65 2	6.96 $\pm$ 0.22 3
Visual cortex	4.55 $\pm$ 0.55	5.06 $\pm$ 0.38
Lateral geniculate	5.58 $\pm$ 0.79 2	7.03 $\pm$ 0.15 2
Superior colliculus	6.22 $\pm$ 0.53 2	2.65 $\pm$ 0.35
Auditory cortex	4.30 $\pm$ 0.29	6.99 $\pm$ 0.13 3
Medial geniculate	5.70 $\pm$ 0.70 2	7.05 $\pm$ 0.16 4
Inferior colliculus	6.95 $\pm$ 0.11 4	5.89 $\pm$ 1.02 2
Superior olive	3.31 $\pm$ 1.21 1	1.76 $\pm$ 0.27
3. Hippocampus	2.12 $\pm$ 0.21	3.21 $\pm$ 0.50
Amygdala	3.09 $\pm$ 0.22	3.65 $\pm$ 0.21
Septal nucleus	2.53 $\pm$ 0.13	4.26 $\pm$ 0.28
Habenula	3.29 $\pm$ 0.34	4.91 $\pm$ 0.33
4. Hypothalamus	4.51 $\pm$ 0.41	4.70 $\pm$ 0.37

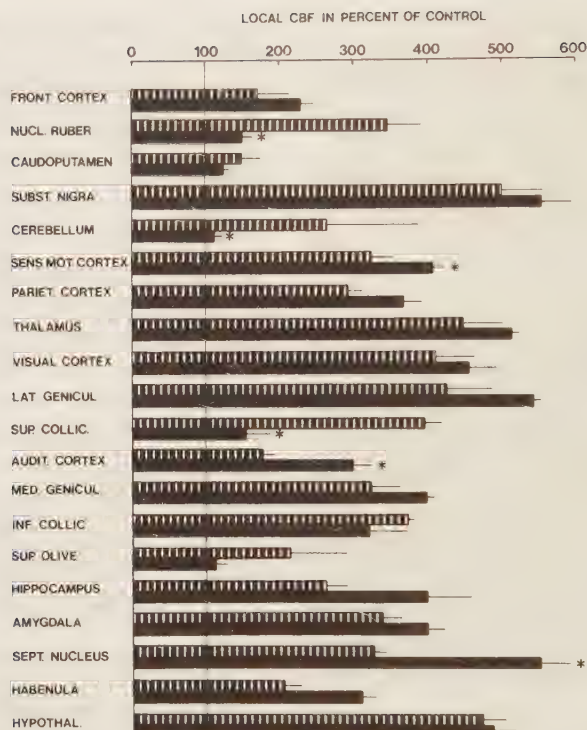


Fig. 1. l-CBF in per cent of control in the bicuculline group (hatched) and the bicuculline + indomethacin group (black). The asterisks indicate significant differences ($P < 0.05$) between the two groups.

transiently approached 180 mmHg but results in this animal did not differ from those obtained in the others. In all animals P_{aO_2} exceeded 85 mmHg and arterial pH was 7.20, or higher, at the time of l-CBF determination. As Table 1 shows body temperature, mean arterial blood pressure and arterial P_{CO_2} were similar in the two experimental groups.

Table 2 gives means $\pm$ S.E. for l-CBF in 21 structures in the brain in the two groups. In order to facilitate inspection of data and to provide comparisons with control CBF values the l-CBF figures were calculated in per cent of control (Fig. 1). In the figure and the table the structures were arranged in the following groups: (1) "motor", (2) "sensory", (3) "limbic", and (4) hypothalamus. We will consider in turn the distribution of l-CBF values during seizures and the modulating influence of indomethacin.

Distribution of l-CBF during seizures

The percentage changes in l-CBF are shown in Fig. 1 (hatched bars). As can be seen, the increase in CBF within motor structures was quite variable. Thus, whereas marked increases were seen in substantia nigra, nucleus ruber and cerebellum, CBF increased only moderately in the frontal cortex and hardly at all in the caudoputamen. Among the sensory structures, marked increases in flow rates ($>300\%$ of control) were observed in all except the auditory cortex and the superior olive (about 200% of control). All limbic structures showed moderate to marked increases (200–350% of control). Finally, an excessive increase occurred in the hypothalamus (to about 470% of control). In conclusion, bicuculline-induced seizures are accompanied by markedly increases flow rates in the majority of cerebral structures visualized by the

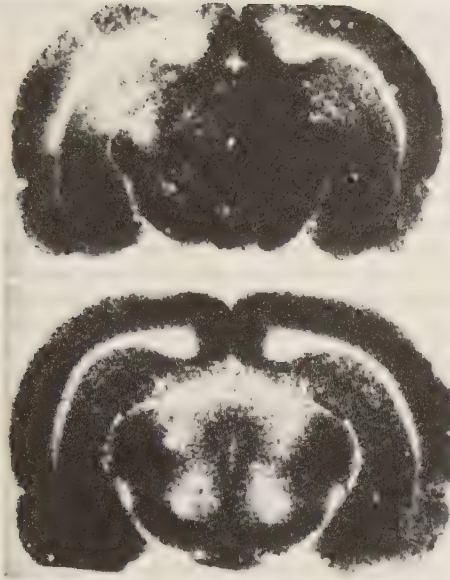


Fig. 2. Autoradiograms at the level of nucleus ruber showing the differences between a bicuculline injected animal (top) and an animal injected with bicuculline and indomethacin (bottom).

present autoradiographic technique but some provide notable exceptions (see Discussion).

The effect of indomethacin

In general the present results show that administration of indomethacin has small effects on the increase in CBF during seizures. The results, nevertheless, suggest that indomethacin influences CBF during seizures in some brain structures. For example, the drug virtually abolished the increase in CBF in the cerebellum, nucleus ruber, superior colliculus (Fig. 2), and superior olive (in the latter structure, one aberrant value in the uninjected group gave an unusually large standard error, see Table 1). However, an opposite effect was observed in the septal nucleus and in the sensory motor and auditory cortices. These data demonstrate that inhibition of prostaglandin synthesis has complex effects on the circulatory response during seizures (see Discussion).

DISCUSSION

The present results which give novel information on cerebral circulation during generalized seizures will

be discussed under the headings of the results section.

Distribution of I-CBF during seizures

Previous results on cerebral blood flow during bicuculline-induced seizures in rats, obtained with a technique that predominantly measures cortical flow rates, demonstrate a 4-fold increase after 20 min of seizures activity with a 2- to 3-fold increase in cerebral oxygen consumption (CMR_{O_2}) (Meldrum & Nilsson 1976). In the present study, CBF in parietal and sensori-motor cortex increased to 325 % of control. If one takes the difference in mean arterial blood pressure into account (about 20 mmHg) the agreement is quite satisfactory. Meldrum & Nilsson (1976) failed to find that phen-tolamine influences the circulatory or metabolic response during seizures, once the initial pressure peak had subsided. We thus concluded that phen-tolamine, as used presently, did not significantly alter cerebral circulation after 20 min of seizure activity.

The present results are not directly comparable to those reported by Mueller et al. (1979), who measured CBF in cats that had a 70 mmHg increase in mean arterial blood pressure. Besides, their technique allowed measurements on only a few grossly dissected brain structures. However, the present results confirm their finding that CBF in cerebral cortex and "thalamus-midbrain" increased more than CBF in the cerebellum.

Since Horton et al. (1980) found that the microsphere technique underestimated CBF during seizures, and sometimes gave differences between the hemispheres, we will only consider their ^{14}C -iodoantipyrine data. In their study, the 30 min group had a mean blood pressure similar to that of our own group, hence the results can be compared. For many of the structures the percentage increases in the two studies are comparable. However, the results differ for some others. For example, whereas Horton et al. (1980) found similar increases in anterior, middle and posterior cortex our data show large differences in percentage increase in CBF between different cortical structures. Furthermore, since many of the control CBF values reported by Horton et al. (1980) differ considerably from our own many of the absolute CBF values during seizures are discordant. Further work is required to elucidate the cause(s) of the differences observed. Quantitatively determination of CBF with the

technique of Landau et al. (1955) requires that flow is homogenous in the structure examined and this requirement can only be fulfilled with autoradiographic evaluation of ^{14}C -activities in tissue (Kety 1960, see also Eklöf et al. 1974). Furthermore, at high flow rates great effort has to be made in determining the arterial curve without distortion (see Methods).

Our results provide the first account of changes in local CBF during seizures induced by the GABA receptor blocker bicuculline. The results demonstrate that the increase in I-CBF is markedly heterogeneous with increases in flow rates ranging from 150 to 500% of control. In fact, since some I-CBF values only represent estimates of minimal flow rates (see Methods and Table 2) the range must be even larger.

With two exceptions (auditory cortex and superior olive, both processing auditory stimuli) all "sensory" structures showed a fairly homogeneous increase in I-CBF (325–450% of control). A fairly homogeneous CBF response was also observed in limbic structures (CBF 200–340% of control). There was a striking 5-fold increase in CBF in the hypothalamus, and a marked heterogeneity in "motor" structures. Among the latter the increase in CBF ranged from 150% (caudoputamen) to 500% of control (substantia nigra).

The cause of variability in CBF response is at present unknown. The distribution of flow rates does not seem to correlate with the corresponding distribution of GABA receptor sites (Fahn & Coté 1968, see also Mueller et al. 1979). A comparison with local CBF values in control animals (Abdul-Rahman et al. 1979, Dahlgren et al., in preparation) reveals no relationship with the relative increase during seizures. For example, the moderate relative increase in CBF in auditory cortex cannot be due to its high resting CBF since an equally moderate (relative) increase occurred in structures with a much lower resting CBF (e.g. hippocampus). Furthermore, other "high flow" structures such as the inferior colliculus showed a much larger relative increase in CBF than the auditory cortex. We conclude that the distribution of flow rates during seizures in a complex way reflects differences in functional activity. For further elucidation of mechanisms involved information is urgently warranted on the corresponding changes in local metabolic rate during seizures (cf. Siesjö & Abdul-Rahman 1979) as well as on the distribution of

flow rates in other "high flow" conditions such as hypercapnia and hypoxia.

The influence of indomethacin

The present results should be considered in relationship to those demonstrating a pronounced influence of indomethacin on CBF in normocapnic as well as in hypercapnic animals. Results in rats exposed to the same operative and anesthetic conditions as the present ones demonstrate that indomethacin reduces (cortical) CBF to 50% of control and that the drug virtually abolishes the circulatory response to hypercapnia (Sakabe & Siesjö 1979, see also Pickard & MacKenzie 1973 for results on baboons). Of particular interest is the fact that CBF in normocapnic and hypercapnic animals given indomethacin decreases in a relatively homogeneous manner (Dahlgren et al., in preparation).

The circulatory effects of indomethacin under normocapnic and hypercapnic conditions (Pickard & MacKenzie 1973, Sakabe & Siesjö 1979) have not been observed by all. Thus, Wei et al. (1980) recently reported that indomethacin administration to cats ($3 \text{ mg} \cdot \text{kg}^{-1}$) failed to alter resting vascular tone or the response to hypercapnia. These authors suggested that the effects observed by Pickard & MacKenzie (1973) were due to alkalization of cerebral extracellular fluids by bicarbonate in the fluid used for infusion. In view of the fact that Sakabe & Siesjö (1979) dissolved the indomethacin in physiological saline, this explanation seems less likely. Furthermore, in that study indomethacin had a pronounced effect on the circulatory response to hypercapnia but not to hypoxia. We conclude that in the monkey and the rat the effects of indomethacin are unequivocal. It remains to be settled whether the results reported by Wei et al. (1980) were due to the fact that they measured pial vascular diameter rather than CBF, or if the barbiturate anaesthesia used influenced the results.

Several types of experimentally induced seizures have been found to be accompanied by increased formation of prostaglandin in the brain (Folco et al. 1977, Marion & Wolfe 1978, Steinhauer et al. 1979). It has also been established that indomethacin will block this increase (Steinhauer et al. 1979).

The present results unequivocally demonstrate that in the majority of the structures visualized in the present study indomethacin did not curtail the increase in CBF. It may, therefore, be concluded

that, in contrast to hypercapnia, bicuculline-induced seizures increase CBF by mechanisms that are unrelated to formation of prostaglandins or related substances. However, it must be emphasized that there were notable exceptions. Thus, in the indomethacin-treated animals the reduction of CBF in the nucleus ruber, cerebellum, superior colliculus, and superior olive is similar to that observed during hypercapnia. Any attempted explanation must at present be speculative. However, it seems urgently warranted to evaluate the effect of indomethacin on local metabolic rate. Furthermore, since localized tissue edema may well influence cerebral vascular resistance, and since metabolites of arachidonic acid, the precursor of prostaglandins, thromboxanes and prostacyclin, may precipitate edema (see Chan & Fishman 1978, Flower 1979) analyses of water content and histopathologic alterations are required. Possibly, such analyses may also provide clues to the possibility that indomethacin increases I-CBF in some structures during seizures (see Fig. 1). However, since an increased sympathetic discharge somewhat curtails the increase in CBF during seizures (Mueller et al. 1979) an interaction between biogenic amines and prostaglandins should also be considered as a possibility (Hedqvist 1977).

As stated in the introduction there is meagre evidence that products of an increased metabolic rate such as H^+ and adenosine mediate the increased CBF during seizures. The present results demonstrate that prostaglandins do not seem to provide the missing coupling factor. Thus, the mechanisms leading to an increased CBF during paroxysmal neuronal activity remain elusive.

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LOCAL BLOOD FLOW AND GLUCOSE CONSUMPTION IN THE RAT BRAIN
DURING SUSTAINED BICUCULLINE-INDUCED SEIZURES

Martin Ingvar and Bo K. Siesjö

ABSTRACT

Local cerebral blood flow and glucose utilization were measured autoradiographically in ventilated rats, in which seizures of 20, 60, or 120 min duration were induced by i.v. bicuculline.

After 20 and 60 min of seizure activity local CBF increased 2- to 4-fold in most of the 21 structures analysed. However, there was a marked heterogeneity with CBF values varying between 150% (caudoputamen) and 500% (globus pallidus) of control. After 120 min CBF values in several structures, notably cortical and limbic regions, fell in spite of unchanged blood pressure and continued seizure activity.

Changes in local CMR_{gl} were equally heterogenous, and correlated poorly with blood flow rates. Some structures (the cerebral cortices and three limbic areas) showed a sustained 2-4 fold increase in CMR_{gl} . In these, metabolic rate and blood flow were initially matched but CBF subsequently fell to yield a pattern of relative hypoperfusion. Other structures showed no, or only moderate, increases in CMR_{gl} . In spite of this, CBF increased markedly to yield a pattern of relative hyperemia. It is concluded that bicuculline induced seizures represent a condition in which structures, traditionally assumed to be prone to develop cell damage, show grossly enhanced metabolic rate and develop relative underperfusion. Furthermore, the results suggest that the seizures induced lead to a striking mismatch between local metabolic rate and blood flow.

INTRODUCTION

Epileptic seizures are accompanied by marked increases in cerebral metabolic rate and blood flow and, if recurrent or prolonged, they may give rise to neuronal cell damage (for literature, see Corsellis and Meldrum 1976, Siesjö 1978). Such damage may be caused by the excessive neuronal activity per se and/or by a mismatch between oxygen supply and oxygen demand. It is natural, therefore, that efforts have been made to estimate cerebral metabolic rate for oxygen ($CMRO_2$) and glucose (CMR_{gl}), as well as cerebral blood flow (CBF), and to attempt relating changes in these variables to histopathological alterations.

At present, much information is available on models in which local seizures have been induced by penicillin, by kainic acid, or by

lidocain (see discussion). However, the most extensively studied model is that in which global seizures are induced by i.v. bicuculline, a GABA receptor blocker. Although the model has been used to assess neuronal cell damage in the baboon (Meldrum and Erierly 1973), and the cat (Brown et al 1980), the most comprehensive data exist for the rat. In that species, information is at hand on seizure-induced changes in CBF, CMR_{O_2} , and CMR_{gl} (Meldrum and Nilsson 1976, Borgström et al 1976, Elenow et al 1979), in tissue metabolites (Chapman et al 1977, Elenow et al 1977, 1979, Folbergrová et al 1981, Siesjö et al 1982a), and in cell structure (Elenow et al 1978, Söderfelt et al 1981). These studies have shown that although the phosphorylation state of the cerebral tissues remains only moderately perturbed, signs of neuronal cell damage nevertheless appear after 1-2 hours of continuous seizure activity. However, in ventilated and well oxygenated fed animals this damage is mainly confined to the cerebral cortex and the hippocampus while the cerebellar Purkinje cells, traditionally assumed to be vulnerable, are spared.

In view of the preferential localization of neuronal cell damage to certain regions it is of interest to obtain information on regional changes in CBF and metabolic rate. Current information is incomplete. Thus although Siesjö and Abdul-Rahman (1979), using the ^{14}C -deoxyglucose technique of Sokoloff et al. (1977), reported that CMR_{gl} during bicuculline-induced seizures was markedly increased in cerebral cortex and hippocampus, but not in the cerebellum, the results only apply to 20 min of seizure activity, and a quantitative analysis of CMR_{gl} in different brain structures was not attempted. Furthermore, although Horton et al. (1980) recently published results on local CBF, no data on metabolic rate were reported. Besides, since the CBF results were based on dissection of tissue regions (for ^{14}C -iodoantipyrine measurements) the data are not directly comparable to those obtained with quantitative autoradiography.

In the present study, we induced bicuculline seizures in ventilated rats, and measured local CBF and CMR_{gl} after 20, 60, and 120 min of continuous seizure activity, using autoradiographic techniques. Although CBF and CMR_{gl} were measured in separate animals, the procedures were so standardized that the results should be directly comparable. During the course of the study it became clear that, with prolonged seizures, the CMR_{gl} values of some structures

were considerably higher than those predicted from previous data on "cortical" $CMRO_2$ and CMR_{gl} . The discrepancy seemed to be caused by a change in the "lumped" constant (see Sokoloff et al. 1977), used to calculate local CMR_{gl} . Attempts were therefore made to derive more appropriate constants from plasma and tissue glucose concentrations. Preliminary accounts on some of the present results have been published (Ingvar and Siesjö 1981, Siesjö et al. 1982a).

METHODS AND MATERIALS

Materials

Bicuculline methachloride (Sigma) was dissolved in 0.1 N HCl and brought to a pH of about 5 with 0.1 N NaOH and then diluted with Krebs-Hensleit solution to yield a concentration of $1.2 \text{ mg} \cdot \text{ml}^{-1}$. ^{14}C -iodoantipyrine (Amersham) was delivered in ethanol solution with a specific activity of $54 \text{ mCi} \cdot \text{mol}^{-1}$. Before each experiment the ethanol was evaporated and the tracer dissolved in 0.5-0.7 ml of Krebs-Hensleit solution to yield an activity of $50 \text{ } \mu\text{Ci} \cdot \text{ml}^{-1}$. ^{14}C -2-d-deoxyglucose (Amersham) with a specific activity of $230 \text{ mCi} \cdot \text{mmol}^{-1}$ was delivered in an ethanol-containing water solution. An aliquot was evaporated before each experiment and redissolved in 0.7 ml Krebs-Hensleit solution. $^{133}\text{Xenon}$ was obtained from Studsvik (Stockholm).

Animals, operative and sampling techniques

Male Wistar rats of a SPF strain (Møllegaard Avlslaboratorium, Copenhagen), weighing 310 to 400 g, were used. A previous study from the laboratory has shown that when bicuculline is injected into fasted animals the seizure discharge (and metabolic rate) is attenuated after 5-15 min, concomitant with a decrease in blood glucose concentrations below about $3 \text{ } \mu\text{mol} \cdot \text{g}^{-1}$ (Blennow et al. 1979). The experiments were therefore performed on animals fed rat pellets (Astra-Ewos, Södertälje, Sweden) ad libitum. One consequence of this was that plasma glucose concentrations were very high during the seizure periods employed (see below).

The animals were anesthetized in a jar with a gas mixture containing 3% halothane and 65% nitrous oxide in oxygen. When unresponsive, they were tracheotomized, connected to a Starling type respirator (Braun, Melsungen), paralyzed with tubocurarine ($0.5 \text{ mg} \cdot \text{kg}^{-1}$ i.v.) and ventilated on 1% halothane and 70% nitrous oxide in oxygen until the end of the operative procedures. Body temperature,

as measured in the rectum, was kept close to 37°C by means of a heating bulb. One femoral artery was cannulated for continuous blood pressure recording (Siemens Elema connected to a Statham transducer), and for sampling of blood for analyses of blood gases, and pH (Eschweiler, Kiel, and Radiometer, Copenhagen), as well as whole blood and plasma glucose concentration. Both femoral veins were cannulated for infusion of drugs, blood (when necessary), glucose, and radioactive tracer. Holes were drilled in the skull and gold-plated screws were fastened for recording of the electroencephalogram with bipolar leads (Siemens Elema, Stockholm). Additional procedures for the different series of animals were as follows:

1-CBF: A short (maximally 20 mm) catheter was inserted in the brachial artery on one side in order to obtain arterial ^{14}C -activity estimates with minimal smearing and time lag (see Dahlgren et. al. 1981a, Ingvar et al 1981). The animals were placed in a guillotine for simultaneous decapitation with the last arterial sample.

1-CMR_{gl}: The femoral artery, not used for measurements of the blood pressure, was used for arterial sampling in order to obtain plasma glucose concentration and ^{14}C -activity.

CBF and CMRO₂: In these animals, preparations were made for sampling of cerebral venous blood from the superior sagittal sinus, allowing estimation of venous oxygen content and $^{133}\text{Xenon}$ activity.

Tissue glucose concentrations. In these animals, a skin incision was placed over the skull bone to accomodate a plastic funnel for later freezing of the brain in situ.

Upon the completion of the operative procedures, the halothane supply was discontinued and the animals were ventilated on 70% nitrous oxide in oxygen for a least 30 min before the experiment was started. During that time the temperature of the animals was kept close to 37°C and the arterial PO₂ and PCO₂ were adjusted to above 90 mmHg and in the range 35-40 mmHg, respectively.

Induction and maintenance of seizures: At the end of the 30 to 40 min steady state period 3-5 ml blood was slowly withdrawn so as to reduce mean arterial blood pressure from 140-150 mmHg to 95-105 mmHg (this was done in order to reduce the ictal increase in pressure at the onset of seizures, see Meldrum and Nilsson 1976, Chapman et al 1977). Bicuculline was then injected i.v. in a dose of 1.2 mg.kg⁻¹. In all experiments, the initial pressure rise was followed by a slow

decrease in blood pressure. In order to maintain mean arterial blood pressure at 110 to 120 mmHg, the blood withdrawn plus donor blood was reinfused at later stages of the seizure period as needed.

Experimental groups

The series devoted to l-CBF measurements included a control group and three experimental groups in which ^{14}C -iodoantipyrine was infused after 20, 60, and 120 min of continuous seizure activity. Comparable groups were used for measurements of l-CMR_{gl}. In these, ^{14}C -deoxyglucose was infused after 20, 45, and 105 min of seizure activity, and sampling of blood was continued for an additional 40 min period before the animals were decapitated. Henceforth, these groups will be denoted as the 20, 60, and 120 min l-CMR_{gl} groups. For reasons given below, yet another l-CMR_{gl} group was studied. In that, the animals were infused with glucose i.v. ($1 \text{ ml} \cdot \text{h}^{-1}$ of a 50% glucose solution) during the last 60 min of seizure activity. Measurements of the CBF-CMRO₂ couple were performed after 2 hrs of seizures in animals that were not infused with glucose. Finally, regional glucose concentrations were measured in three control animals, in three with 20 min, and in two with 120 min of seizure activity.

Methods and analytical techniques

Local-CBF: l-CBF was measured autoradiographically with ^{14}C -iodoantipyrine as the diffusible tracer (Sakurada et al 1978). Details of the procedures have been given in a previous communication (Abdul-Rahman et al 1979). A few modifications were made, namely: 1) the blood was sampled from the brachial artery, 2) the sampling time was reduced to 20 sec in animals with seizures, and 3) a small error in the calibration was corrected. For further information on the modifications, see Dahlgren et al (1981a). In summary, the isotope was infused at a constant rate i.v. during a period of 20 sec while arterial blood was sampled every 2 sec (in controls these times were set to 45 and every fourth sec, respectively). At the end of the infusion period, simultaneous with the last blood sample, the animals were decapitated, the brain was removed and within two and a half minutes immersed in isopentane cooled to -55 to -70°C with liquid nitrogen. The ^{14}C -activity in the blood was measured with liquid scintillation counting, the counting efficiency being determined with ^{14}C -hexadecane as an internal standard. The radioactivity of the brain regions was determined with quantitative autoradiography. The brain

was cut into 20 μm thick slices, and the slices, together with a set of calibrated ^{14}C standards, were applied to a single layer X-ray film (Kodak SB-5). The exposure time was one week. The optical density of the autoradiograms was evaluated manually with a MacBeth (Td 501) transmission densitometer with an aperture of 1 mm. Four control animals in the l-CBF series are identical to those in a previous communication (Lindvall et al 1981) as the two studies were carried out simultaneously.

Local-CMR_{gl}: For measuring l-CMR_{gl} quantitative autoradiography was used according to Sokoloff et al (1977). Details of the procedure have been given in a previous communication (Ingvar et al 1980). In brief, a solution with 15 μCi (seizure animals) or 50 μCi (controls) of ^{14}C -deoxyglucose was infused i.v. over a period of 30 seconds. Repeated arterial samples of about 50 μl were taken for analysis of ^{14}C -activity (beta scintillation-counting) and glucose concentration (hexokinase method or a calibrated automatic Beckman glucose analyzer). After the 40 min steady state period the animals were decapitated and the brains were analyzed with quantitative autoradiography as described above.

CBF and CMRO₂. For measuring "cortical" CBF and CMRO₂ we used the Kety-Schmidt technique (1948) with $^{133}\text{Xenon}$ as described in previous communications from the laboratory (Norberg and Siesjö 1974, Berntman et al. 1979). The animals were saturated with $^{133}\text{Xenon}$ for 20 min before desaturation was started and repeated arterial and cerebral venous blood samples were collected for determination of $^{133}\text{Xenon}$ activities and arteriovenous differences in oxygen content.

Regional glucose concentrations. Following freezing of the brain in situ (Pontén et al. 1973) and storage of tissue at -85°C , the brains were dissected at -22°C . Samples (10-20 mg) representing parietal, sensorimotor and occipital cortex, thalamus, hippocampus, hypothalamus, colliculus inferior, and cerebellum were weighed and extracted with HCl-methanol at -22°C , and subsequently handled at 0°C . Further processing of the samples, and the method for measuring glucose concentrations, have been described previously (see Folbergrová et al. 1981).

Calculations

Local CBF was calculated as described by Sakurada et al. (1978) and CBF-CMRO₂ according to Norberg and Siesjö (1974). For calculating

1-CMR_{gl} in all structures of control and seizure groups we employed the equation described by Sokoloff (1977). However, as will be described below we subsequently corrected the lumped constant, using information on blood and tissue glucose concentrations and the nomogram constructed by Pardridge et al. (1982 a,b). Statistical differences between groups were calculated with the unpaired Student's t-test. $p < 0.01$ was chosen as the level of significance in differences.

RESULTS

Changes in Physiological Variables

There were four series of experimental animals, used for studying 1-CBF, 1-CMR_{gl}, CBF-CMRO₂, and regional glucose concentrations. In all of the animals belonging to these series, the bicuculline injection gave rise to the expected seizure activity and, in all, the seizure pattern was as described in previous communications (Meldrum and Nilsson 1976, Chapman et al 1977). Thus, following a burst of paroxysmal activity a pattern of burst and suppression was maintained until termination of the experiments (see below).

Table 1. Physiological parameters in the various experimental groups.

	temp C	MABP mmHg	PeakBP mmHg	PCO ₂ mmHg	PO ₂ mmHg	pH

local cerebral blood flow:						
control	36.8+0.2	144+5	-	37.9+1.6	99+3	7.41+0.03
20 min seizures	36.5+0.2	116+2	198+6	26.5+1.5	110+4	7.15+0.03
60 min seizures	37.0+0.2	116+2	205+7	35.7+1.4	106+4	6.94+0.04
120 min seizures	36.4+0.1	113+2	196+6	33.8+1.5	98+4	6.97+0.06
regional cerebral blood flow:						
120 min seizures	36.7+0.2	112+4	184+11	35.9+0.8	116+2	7.10+0.10
local cerebral glucose utilization:						
control	37.1+0.2	139+8	-	37.4+0.7	112+3	7.39+0.01
20 min seizures	37.0+0.1	116+1	198+4	32.4+2.1	117+3	6.91+0.02
60 min seizures	37.1+0.3	111+1	205+7	36.6+1.1	109+6	7.02+0.05
120 min seizures	37.5+0.2	115+2	179+3	36.0+0.8	107+2	7.17+0.03
120 min seizures+gluc.	37.4+0.2	110+2	185+4	34.9+1.5	100+7	7.23+0.05

Values are given as means + S.E. MABP=mean arterial blood pressure
Peak BP=Mean peak blood pressure following bicuculline injection.

Body temperature, mean arterial blood pressure, peak mean pressure after injection of bicuculline, as well as arterial PO_2 , PCO_2 , and pH in the control and experimental groups are given in Table 1. The physiological data obtained were similar to those previously described for bicuculline-induced seizures (Meldrum and Nilsson 1976, Chapman et al 1977, see also Blennow et al 1979). In all groups with seizures the body temperature was sufficiently similar to control values to exclude the possibility that temperature variations could have influenced the results. All animals with seizures had similar peak arterial blood pressures and final mean arterial pressures below control values. However, the seizure groups had similar mean arterial blood pressures. In the l-CBF groups, variations in blood pressure during the last 5-10 min were within 10% of the final values. All animals had arterial PO_2 values exceeding 90 mmHg. All groups except two had $PaCO_2$ values in the range 34-38 mmHg. In the initial 20-60 min period, though, $PaCO_2$ values were lower (cf. Meldrum and Nilsson 1976, Chapman et al. 1977). The seizure state was accompanied by marked plasma acidosis.

Local CBF

In measuring local CBF, we paid particular attention to two factors of potential importance for the accuracy of results. First, since the l-CBF technique integrates flow over a period of only 20 sec, it seemed essential to compare the seizures discharge in individual animals. Second, since seizures must be expected to impair

vascular autoregulation it was carefully controlled that blood pressure did not vary unduly before or during the CBF measurements. In one 20 min animal the blood pressure fell by 20 mm Hg during the flow measurement and the animal was therefore excluded from the material Fig. 1 illustrates

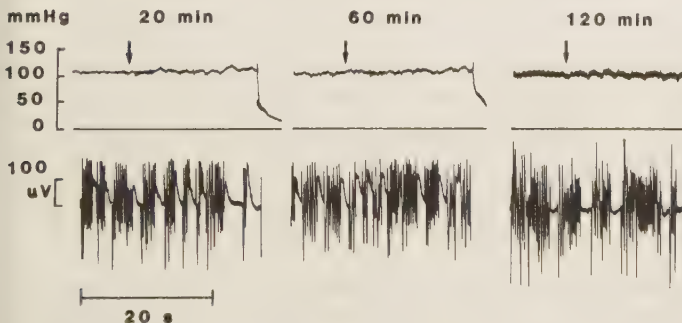


Figure 1. Blood pressure and EEG recordings in three animals during and before the measurement of l-CBF, one from each experimental group. The arrow indicates the start of the ^{14}C -iodo-antipyrine infusion.

that blood pressure could be maintained constant during the 20 sec sampling period and that the burst and suppression pattern was upheld until the decapitation of the animals. In each animal, the "silent" periods (i.e. the periods in which bursts or spikes were absent) were calculated in per cent of the total (20 sec) periods. In the 20, 60, and 120 min groups, the figures obtained were 33 $\pm$ 4, 20 $\pm$ 2, and 29 $\pm$ 3 per cent, respectively. Thus, seizure activity seemed somewhat more "intense" at 60 than at 20 min ($p < 0.05$). Most important, though, was the fact that the seizure discharge was not attenuated after 120 min ($p > 0.05$, as compared to 20 and 60 min).

Table 2 gives results on l-CBF after 20, 60, and 120 min of continuous seizure activity. In the 20 min group all structures

Table 2. Local cerebral blood flow in rats subjected to bicuculline induced seizures .

	control n=5	20 min n=6	60 min n=8	120 min n=7
Frontal cortex	1.51 $\pm$ 0.14	3.20 $\pm$ 0.23 *	2.60 $\pm$ 0.18 *	1.79 $\pm$ 0.13
Nucleus ruber	1.15 $\pm$ 0.12	4.11 $\pm$ 0.39 *	4.11 $\pm$ 0.31 *	2.51 $\pm$ 0.14 *
Substantia nigra	0.93 $\pm$ 0.10	3.81 $\pm$ 0.27 *	2.92 $\pm$ 0.17 *	1.32 $\pm$ 0.37
Caudoputamen	1.19 $\pm$ 0.14	1.76 $\pm$ 0.10 *	2.12 $\pm$ 0.23	1.73 $\pm$ 0.24
Globus pallidus	0.58 $\pm$ 0.11	2.75 $\pm$ 0.15 *	2.12 $\pm$ 0.23 *	1.38 $\pm$ 0.06 *
Cerebellum	0.80 $\pm$ 0.08	1.37 $\pm$ 0.15	2.17 $\pm$ 0.21 *	1.73 $\pm$ 0.20 *
Sens.mot. cortex	1.56 $\pm$ 0.12	4.27 $\pm$ 0.33 *	4.09 $\pm$ 0.47 *	2.70 $\pm$ 0.21 *
Parietal cortex	1.47 $\pm$ 0.16	4.43 $\pm$ 0.33 *	3.81 $\pm$ 0.38 *	2.39 $\pm$ 0.17 *
Thalamus	1.48 $\pm$ 0.22	5.26 $\pm$ 0.31 *	5.84 $\pm$ 0.38 *	2.91 $\pm$ 0.37
Visual cortex	1.11 $\pm$ 0.16	3.45 $\pm$ 0.18 *	3.29 $\pm$ 0.38 *	2.11 $\pm$ 0.34
Lat. genic. body	1.18 $\pm$ 0.11	4.51 $\pm$ 0.29 *	4.83 $\pm$ 0.35 *	3.05 $\pm$ 0.19 *
Sup colliculus	1.14 $\pm$ 0.13	4.42 $\pm$ 0.28 *	3.89 $\pm$ 0.37 *	3.10 $\pm$ 0.22 *
Auditory cortex	2.28 $\pm$ 0.18	3.70 $\pm$ 0.24 *	4.05 $\pm$ 0.37 *	2.31 $\pm$ 0.17
Med. genic. body	1.53 $\pm$ 0.10	4.84 $\pm$ 0.30 *	5.08 $\pm$ 0.47 *	3.06 $\pm$ 0.28 *
Inf. colliculus	1.90 $\pm$ 0.23	7.33 $\pm$ 0.53 *	3.33 $\pm$ 0.41	3.61 $\pm$ 0.18 *
Sup. olive	1.46 $\pm$ 0.16	3.89 $\pm$ 0.45 *	2.73 $\pm$ 0.30 *	2.09 $\pm$ 0.31
Hippocampus	0.65 $\pm$ 0.07	1.53 $\pm$ 0.17 *	2.18 $\pm$ 0.21 *	1.14 $\pm$ 0.15
Amygdala	0.82 $\pm$ 0.08	2.70 $\pm$ 0.17 *	3.04 $\pm$ 0.18 *	1.88 $\pm$ 0.13 *
Septal nucleus	0.80 $\pm$ 0.10	3.19 $\pm$ 0.18 *	3.17 $\pm$ 0.28 *	2.02 $\pm$ 0.12 *
Habenula	1.33 $\pm$ 0.12	4.16 $\pm$ 0.25 *	4.41 $\pm$ 0.56 *	2.73 $\pm$ 0.19 *
Hypothalamus	0.84 $\pm$ 0.09	3.22 $\pm$ 0.21 *	3.49 $\pm$ 0.24 *	1.92 $\pm$ 0.17 *

Values are given in ml*g $\pm$ min and represent mean $\pm$ S.E.

*denotes significant difference from control ($p < 0.01$)

Table 3. Plasma glucose concentrations in the animals used for l-CMRgl measurements.

	0 min	1 min	6 min	10 min	20 min	30 min
control	9.1+0.7	9.2+0.8	9.6+0.8	9.9+0.9	10.7+1.0	10.9+1.0
20 min of seizures	25.1+2.6	24.1+2.5	24.4+2.2	24.5+2.1	24.5+2.3	25.2+2.5
60 min of seizures	22.3+1.4	21.4+1.4	20.7+1.5	20.3+1.6	19.6+2.2	17.2+2.0
120 min of seizures	14.7+2.8	14.0+2.7	13.3+2.6	12.8+2.5	11.3+2.5	10.1+2.4
120 min of seizures+ glucose	21.4+3.3	20.8+3.1	20.6+2.8	20.0+2.8	19.4+2.5	18.3+2.1

The values are given as Means + S.E. in $\mu\text{mol/ml}$ of plasma

analyzed , except the cerebellum ($p=0.011$), had CBF values significantly higher than control. However, there were appreciable local variations since the caudoputamen, the cerebellum and the auditory cortex showed values of less than 200% of control and the globus pallidus one of close to 500% of control. In the great majority of structures flow rates had increased 2.5- to 4-fold.

After 60 min of seizures, the pattern of CBF changes was similar and clear changes from the 20 min value were only observed in the inferior colliculus and the cerebellum. After 120 min, though, a decrease in CBF occurred in most structures. As a result, some had flow rates that were not significantly altered from control (frontal cortex, caudoputamen, substantia nigra, thalamus, auditory cortex, visual cortex, superior colliculus, hippocampus, and superior olive). It is noteworthy, though, that the secondary reduction in CBF was small or absent in some subcortical structures (cerebellum, superior colliculus, inferior colliculus and superior olive) and pronounced in most of the cerebral cortical (and limbic) structures.

Local metabolic rates

Table 3 shows the plasma glucose concentrations in the groups used for l-CMRgl measurements, as measured during the first 30 min following infusion of ^{14}C -deoxyglucose. Control plasma glucose

concentrations were around $10 \mu\text{mol} \cdot \text{ml}^{-1}$. The values rose 2.5- and 2-fold after 20 and 60 min of seizures but fell towards control values after 2 hrs. Infusion of glucose during the last 60 min of a 2 hrs seizure period gave plasma glucose values similar to those measured in the 60 min group.

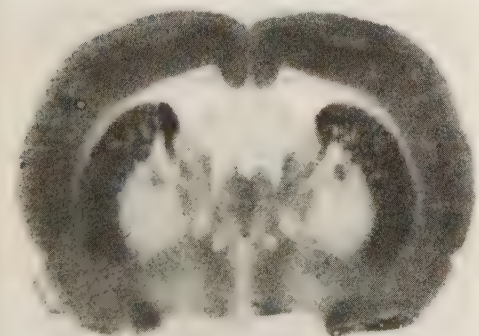
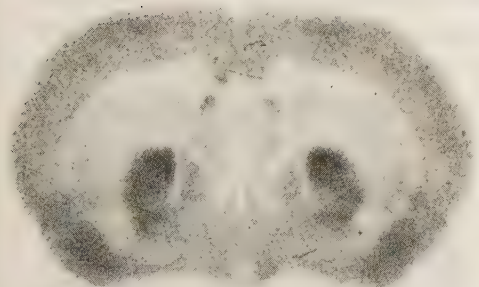
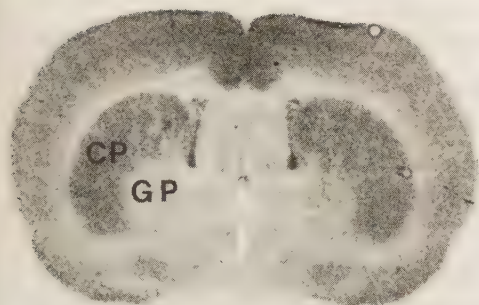
As an initial approach, we calculated 1-CMR_{gl} using a lumped constant of 0.48. The values thus obtained are shown in table 4. After 20 min of seizure activity, the values showed a significant increase in 12 of the 21 structures. In the cerebral cortical structures the values were increased to an average of about 200 % of control (see also substantia nigra, thalamus and hypothalamus). An even larger

Table 4. Local cerebral glucose consumption in the rat subjected to bicuculline induced seizures. All data has been calculated using the standard value for the lumped constant of 0.48.

	Control	20 min	60 min	120 min	120 min +glucose
Frontal cortex	0.74+0.06	1.40+0.08*	2.43+0.17*	3.19+0.16*	2.19+0.24*
Nucleus ruber	0.61+0.05	0.95+0.14	1.07+0.22	0.97+0.24	0.43+0.09
Substantia nigra	0.49+0.03	1.12+0.24	0.34+0.06	0.19+0.15	0.07+0.03*
Caudoputamen	0.82+0.08	1.06+0.08	1.75+0.19*	3.11+0.23*	2.41+0.12*
Globus pallidus	0.39+0.03	1.49+0.29*	0.64+0.17	0.51+0.21	0.26+0.06
Cerebellum	0.43+0.04	0.47+0.09	0.58+0.11	0.76+0.19	0.30+0.06
Sens.mot. cortex	0.65+0.04	1.53+0.11*	2.23+0.13*	3.01+0.09*	2.10+0.15*
Parietal cortex	0.64+0.05	1.46+0.06*	2.25+0.11*	3.08+0.08*	2.04+0.20*
Thalamus	0.86+0.05	1.53+0.13*	2.37+0.24*	2.07+0.15*	1.69+0.20*
Visual cortex	0.73+0.07	1.47+0.13*	2.59+0.15*	2.93+0.08*	2.32+0.15*
Lat. genic. body	0.76+0.06	1.16+0.11	1.65+0.08*	2.23+0.02*	1.45+0.10*
Sup. colliculus	0.67+0.05	1.12+0.14	0.99+0.15	0.98+0.17	0.43+0.08
Auditory cortex	0.85+0.11	1.47+0.07*	2.43+0.13*	3.37+0.09*	2.17+0.13*
Med. genic. body	0.78+0.08	1.27+0.07*	1.42+0.10*	2.31+0.05*	1.45+0.08*
Inf. colliculus	0.83+0.08	0.65+0.10	0.62+0.11	0.75+0.17	0.38+0.08*
Sup. olive	0.54+0.03	0.42+0.13	0.65+0.13	0.65+0.23	0.24+0.07*
Hippocampus	0.52+0.04	1.47+0.09*	2.21+0.33*	2.26+0.16*	1.36+0.27*
Amygdala	0.50+0.03	1.38+0.16*	2.37+0.20*	2.38+0.14*	2.25+0.09*
Septal nucleus	0.40+0.03	1.43+0.13*	2.21+0.17*	2.44+0.10*	1.89+0.05*
Habenula	0.77+0.07	0.64+0.06	0.91+0.14	1.21+0.38	0.77+0.13
Hypothalamus	0.42+0.02	0.93+0.10*	0.97+0.15*	1.36+0.22*	0.69+0.13

Values are given in $\mu\text{mol} \cdot \text{g} \cdot \text{min}$ and represent means+S.E.

*denotes significant difference from control ($p < .01$)



increase (to about 300% of control) was noted in globus pallidus and in three of the limbic structures analysed. In some other structures, finally, the increase in CMR_{gl} was either small or absent (cerebellum, nucleus ruber, caudoputamen, inferior colliculus, superior olive, and habenula).

With a few exceptions, the pattern of changes in $l-CMR_{gl}$ was similar after 60 min. However, it is noteworthy that the absolute values were even higher in cerebral cortical and limbic structures. Clear exceptions to this rule were the globus pallidus and substantia nigra (see also below).

Initially, we performed a series of four animals after 120 min of seizure activity. The results were unexpected since calculated CMR_{gl} in cerebral cortical areas, as well as in the hippocampus and amygdala, increased to values of 400-500% of control. Our previous data (Folbergrova et al. 1981), together with the data of Pardridge et al (1982 a,b) suggested that the calculated values were erroneously high due to a reduction in tissue glucose concentration which induces a rise in the lumped constant (see

Discussion). In order to validate this assumption, CBF and $CMRO_2$ were measured in five animals under otherwise identical conditions. In these, $CMRO_2$ was $9.31 (\pm 1.9 \text{ S.E.M.})$

Figure 2. Deoxy- ^{14}C -glucose autoradiograms at the level of the globus pallidus (GP) and the tail of caudoputamen (CP) in a control animal (top), a 20 min seizure animal (middle), and a two hour seizure animal (bottom).

$\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$. Since a control group of animals yielded a CMRO_2 of $3.52 \pm .22 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ($n=6$) the 120 min seizure values showed that overall cortical metabolic rate had not increased above 300% of control (see Discussion).

In view of these results, another 120 min seizure group was studied with continuous infusion of glucose during the second hour of the seizure period (see Methods, and Table 3). The results of these experiments showed that values for CMR_{gl} in all cerebral cortical and some other structures remained unchanged in the period 60-120 min.

Previous results from the laboratory have given a pictorial illustration of the pronounced differences in density changes between, on the one hand, the cerebral cortex and the hippocampus and, on the other hand, the cerebellum (Siesjö and Abdul-Rahman 1979). Representative autoradiograms can be used to illustrate two other changes. First, as fig. 2 shows prolongation of the seizure period

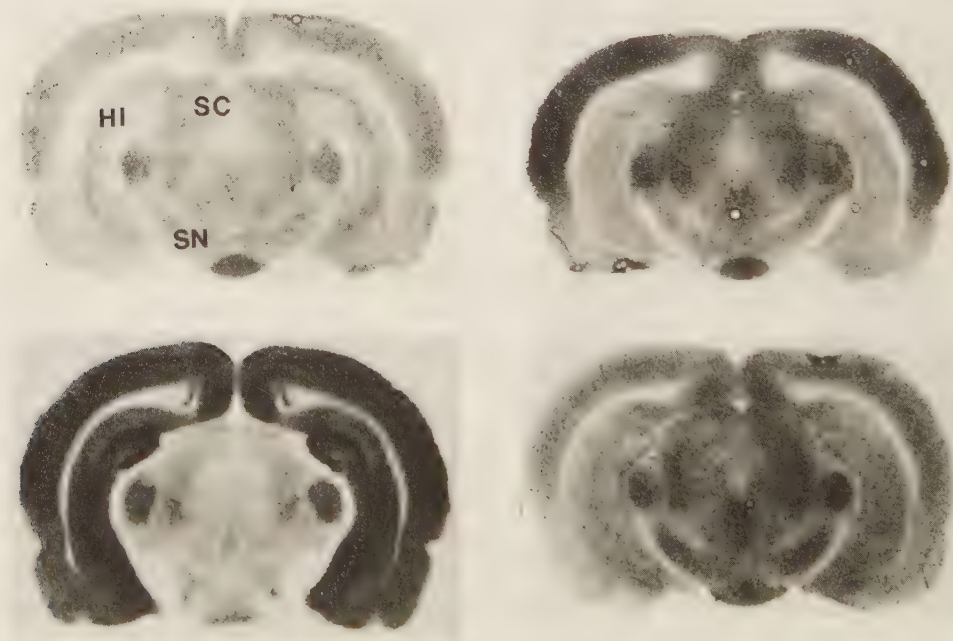


Figure 3. ^{14}C -deoxy-glucose (left) and ^{14}C -iodoantipyrine (right) autoradiograms from control animals (top) and two hour seizure animals. The sections are from the level of the substantia nigra (SN), superior colliculus (SC), and the hippocampus (HI).

from 20 to 120 min gave rise to an increased density in the caudoputamen but to a marked reduction in density in the globus pallidus. Second, fig. 3 illustrates how prolonged seizures lead to a marked reduction of $l\text{-CMR}_{gl}$ in the substantia nigra, without a corresponding fall in $l\text{-CBF}$ (see corresponding dissociation for superior colliculus).

Tissue to blood glucose concentration ratios

Cerebral cortical values for $l\text{-CMR}_{gl}$ in the 60 min animals, and in the glucose-infused 120 min animals, were somewhat higher than those predicted (>300 versus 250% of control, see Discussion). For reasons given below we suspected that this discrepancy, as well as the excessively high values obtained in the non-infused 120 min animals, were due to the fact that the lumped constant varied with the tissue to blood glucose concentration ratios. Such ratios were, therefore, determined in control and seizure animals. As table 5 shows, control animals had tissue to blood glucose concentration ratios of about 0.35 in the majority of regions analysed. After 20 min of seizures, the ratio fell to less than 50% of control in cerebral cortical samples,

Table 5. Blood glucose concentrations and tissue to blood glucose concentrations in control and seizure animals in 8 different brain structures.

	Blood glucose	Tissue to blood glucose concentration ratio							
		PC	SMC	VC	HI	T	HT	CE	IC
control	9.8	0.37	0.48	0.39	0.37	0.35	0.50	-	0.46
"	7.2	0.33	0.41	0.32	0.34	0.36	0.37	0.40	0.34
"	7.9	0.29	0.36	0.33	0.31	0.29	0.40	0.41	0.55
	---	---	---	---	---	---	---	---	---
	8.3	0.33	0.42	0.35	0.34	0.33	0.42	0.41	0.45
seizure 20~	21.8	-	0.19	0.16	0.13	0.15	0.11	0.26	0.20
" 20~	15.0	0.14	0.16	0.15	0.13	0.15	0.15	0.27	0.50
" 20~	16.9	0.10	0.11	0.11	0.07	0.09	0.10	0.23	0.26
" 120~	17.6	0.15	0.13	0.24	0.11	0.25	0.37	0.37	0.43
" 120~	12.0	0.20	0.16	0.13	0.11	0.26	0.31	0.39	-
		---	---	---	---				
		0.15	0.15	0.16	0.11				

PC=parietal cortex, SMC=sensorimotor cortex, VC=visual cortex, HI=hippocampus, CP=caudoputamen, T=thalamus, HT=hypothalamus, CE=cerebellum, and IC=inferior colliculus.

and in the hippocampus , but it fell considerably less in inferior colliculus and cerebellum. Two structures (hypothalamus and cerebellum) showed values indicating an increase in the glucose ratio between 20 and 120 min. In inferior colliculus, the values were too variable to allow firm conclusions regarding seizure-induced changes. The results demonstrate that the glucose ratios (tissue/blood, w/w) cluster around 0.14 for all structures that show values for 1-CMR_{gl} in excess of 200% of control (see table 4 above), and they indicate that the ratios exceed 0.20 in structures that show only a small, or no, increase in 1-CMR_{gl} values (see Discussion).

The CBF/ 1-CMR_{gl} couple

In order to allow an estimate of the coupling of 1-CMR_{gl} and CBF we corrected the 1-CMR_{gl} values given in table 3. As will be described below (see Discussion) this was done by using the information on tissue to plasma glucose concentration ratios and the nomogram published by Pardridge et al. (1982 a, b). As judged from the 1-CMR_{gl} values, three groups of structures could be discerned. In one, comprising the cerebral cortices and three limbic structures (hippocampus, amygdala, and the septal nucleus), the corrected 1-CMR_{gl} values exceeded 200% of control for at least 2 of the 3 time periods studied. In the second group, all 1-CMR_{gl} values were below 200% of control. However, the thalamus was included in that group in spite of the fact that the 60 min value exceeded 200% of control. The third group, finally, consisted of three functionally related structures that displayed marked time-dependent changes in 1-CMR_{gl} (substantia nigra, caudoputamen, and globus pallidus).

Fig. 4 illustrates the percentage changes in 1-CMR_{gl} and CBF in the first group of "hypermetabolic" structures. Certain features were common to all of them. First, with the possible exception of the hippocampus, 1-CMR_{gl} remained grossly elevated when the seizure period was extended from 60 to 120 min. Furthermore, after 20 min of seizure activity the percentage increases in CBF and 1-CMR_{gl} were relatively similar. Finally, prolongation of the seizures to 60 and 120 min was associated with a mismatch between 1-CMR_{gl} and CBF in that a relative hypoperfusion developed. This was especially evident at 120 min when flow rates in many structures decreased towards control values (see also table 3 above).

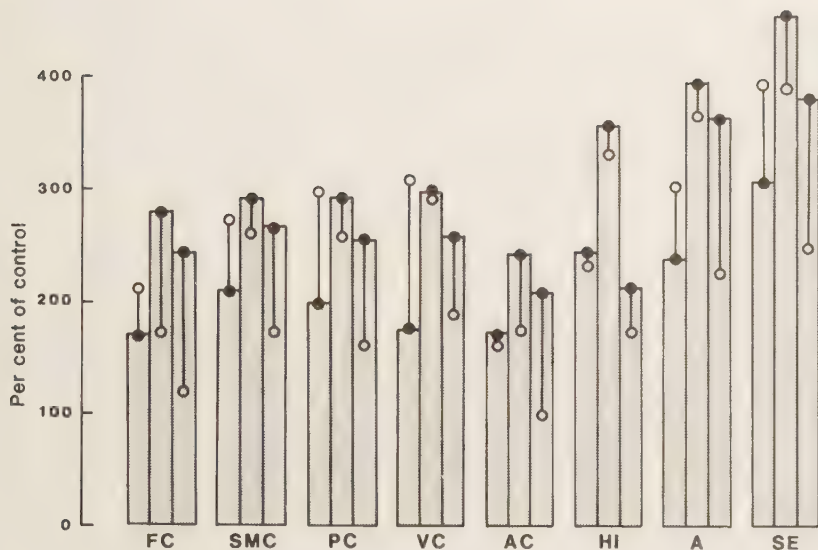


Figure 4. This graph demonstrate the per cent change from control in local glucose utilization (bars with filled circles) and local blood flow (unfilled circles) at 20, 60, and 120 min of seizure activity. FC frontal cortex, SMC sensorimotor cortex, PC parietal cortex, VC visual cortex, AC auditory cortex, HI hippocampus, A amygdala, and SE septal nucleus

The results obtained in the second ("hypometabolic") group are shown in fig. 5. This group was somewhat heterogenous since three structures included (nucleus ruber, thalamus and hypothalamus) had CMR_{gl} values exceeding 150% of control at 2 of 3 times (see also superior colliculus). However, all structures showed a relative hyperemia. Notably, in some structures this was very pronounced, and appreciable increases in CBF occurred in spite of CMR_{gl} values that were close to normal (see especially inferior colliculus, superior olive, and habenula).

The third group of functionally interrelated structures, finally, showed a striking dissociation of changes during the course of seizure activity (fig. 6). Thus, whereas the substantia nigra and the globus pallidus had increased CMR_{gl} values at 20 min, the values subsequently decreased to subnormal values. At all times, though, relative hyperemia persisted. In contrast, CMR_{gl} in the caudoputamen continuously fell, and a relative underperfusion developed. Thus, in terms of the coupling of CMR_{gl} and CBF the globus pallidus resembled

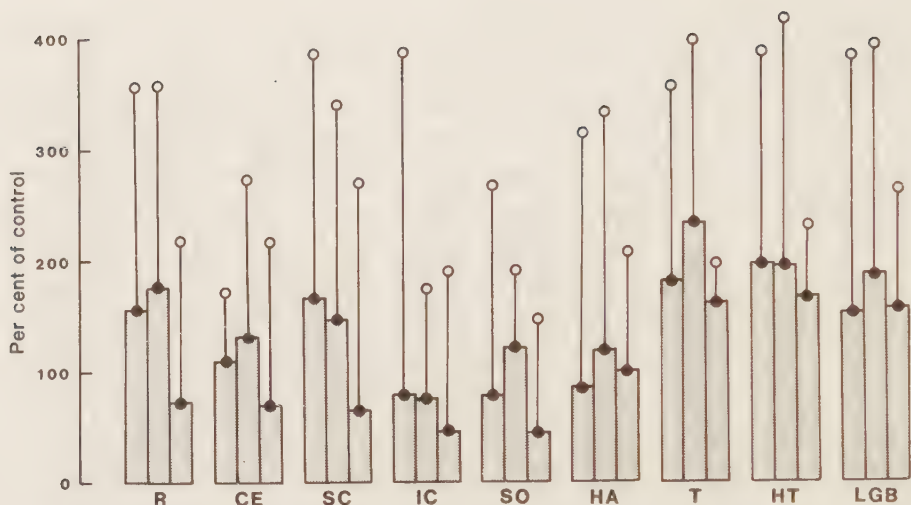


Figure 5. This graph demonstrate the per cent change from control in local glucose utilization (bars with filled circles) and local blood flow (unfilled circles) at 20, 60, and 120 min of seizure activity. R red nucleus, CE cerebellum, SC superior colliculus, IC inferior colliculus, SO superior olive, HA habenula, T thalamus, HT hypothalamus, and LGB lateral geniculate body.

the "hypermetabolic" structures (see fig. 4) while substantia nigra and globus pallidus behaved as the "hypometabolic" ones (see fig. 5).

DISCUSSION

Validity of methods used

Before discussing changes in local CBF and CMR_{gl} during bicuculline-induced seizures, it is necessary consider the methods used. Evidently, the interpretation of the present results is critically dependent on the validity of the l-CBF and l- CMR_{gl} techniques, as applied to epileptic seizures. We submit that the l-CBF technique used, with a short brachial catheter and sampling of blood for only 20 sec, with close control of blood pressure, measures local flow rates that are close to the true ones even when these are markedly increased (see Dahlgren et al. 1981 a).

The ^{14}C -deoxyglucose technique of Sokoloff et al. (1977) has been repeatedly used to measure l- CMR_{gl} in seizure states, notably those induced by penicillin, kainic acid, and lidocain (Collins et al. 1976, Collins 1978, 1980, Kato et al. 1980, Caveness et al. 1980, Lothman and Collins 1981, Ingvar and Shapiro 1981). In all these studies, it has been tacitly assumed that the kinetic and lumped

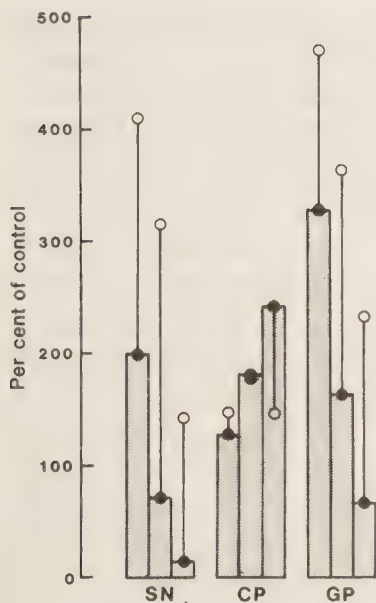


Figure 6. This graph demonstrate the per cent change from control in local glucose utilization (bars with filled circles) and local blood flow (unfilled circles) at 20, 60, and 120 min of seizure activity. SN substantia nigra, CP caudoputamen, and GP globus pallidus.

a reduction in tissue glucose concentration (and thereby in the tissue to plasma glucose concentration ratio) may lead to a pronounced increase in the lumped constant (Crane et al. 1981, Pardridge et al. 1982, a, b). This means that the lumped constant should increase with metabolic rate unless a corresponding enhancement of glucose influx maintains tissue glucose concentrations (Gjedde 1982, Pardridge et al. 1982a). Obviously, seizure states have two opposing influences on the lumped constant. Thus, whereas any accompanying hyperglycemia will tend to decrease the constant, a lowering of the tissue to plasma glucose concentration ratio will tend to elevate it.

Pardridge et al. (1982 a, b) have recently devised an ingenious approach to predict changes in the lumped constant. A nomogram was constructed from determined values for the blood-brain barrier

constants determined for the normal rat by Sokoloff et al. (1977) are applicable to seizure states as well. The critical assumption is that the lumped constant, expressing the ratios of $V_{\max}$ and K_m for glucose and deoxyglucose, a factor correcting for deoxyglucose phosphate hydrolysis ("back reaction"), and the ratio of the apparent distribution volumes of the two sugars, remains unchanged at a value of 0.48. Sokoloff (1981, see also Savaki et al 1980) has emphasized that this is not necessarily true for pathological states, and his group has demonstrated that the lumped constant varies directly with the plasma glucose concentration (Schuier et al 1981, Suda et al 1981). Others have shown that, at very low plasma (and tissue) glucose concentrations, the lumped constant may increase to at least 200% of normal (Crane et al 1981). However, it has been recognized that even if the plasma glucose concentration does not change,

transport constants (K_m , V_{max} , and K_d) for glucose and 2-deoxyglucose, and for the ratio of phosphorylation constants of the two sugars, which allows estimation of the lumped constant from known values of plasma and tissue glucose concentrations. We used the cortex nomogram (Pardridge et al. 1982b) to predict how the lumped constant will change as a function of tissue glucose concentration at "normal" (10

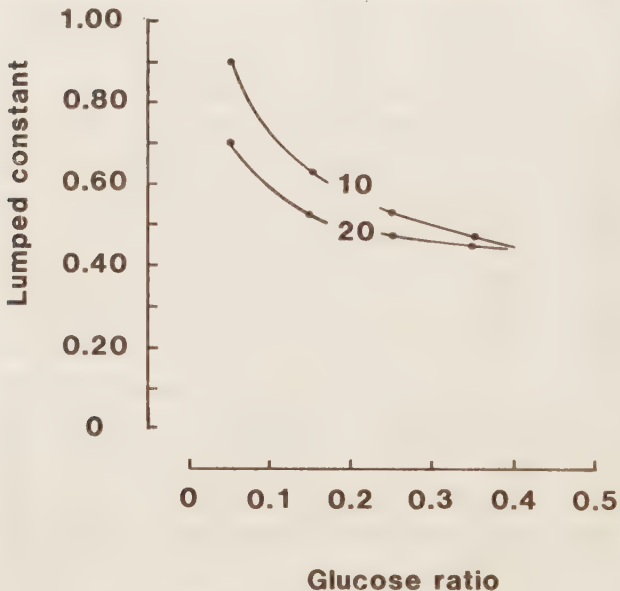


Figure 7. The lumped constant (Sokoloff et al 1977) as a function of the brain tissue to plasma glucose ratio at two different plasma glucose levels. The curves are based on the Pardridge nomogram (1982).

$\mu\text{mol}\cdot\text{ml}^{-1}$) and increased (20 $\mu\text{mol}\cdot\text{ml}^{-1}$) plasma glucose concentration. The values of fig. 7 allow the following conclusions. First, if plasma glucose concentration is maintained at 10 $\mu\text{mol}\cdot\text{ml}^{-1}$ a progressive lowering of the tissue to plasma glucose concentration ratio from 0.40 to 0.05 will lead to an increase in the lumped constant from a control value of about 0.45 to 0.90. Second, although a corresponding increase occurs if the

plasma glucose is maintained at 20 $\mu\text{mol}\cdot\text{ml}^{-1}$, it is clearly less pronounced. Third, if the glucose ratio remains at control values an increase in plasma glucose concentration from 10 to 20 $\mu\text{mol}\cdot\text{ml}^{-1}$ will alter the lumped constant by less than 10%. It follows from these facts that, at a plasma glucose concentration of 20 $\mu\text{mol}\cdot\text{ml}^{-1}$, the lumped constant will remain within narrow limits even if the glucose ratio falls to 0.20, and it is only when the ratio goes below 0.10 that large changes in the lumped constant occur. In other words, the

lumped constant is essentially "clamped" by induced (or spontaneous) hyperglycemia.

Two findings form the basis for our derivation of lumped constants according to Pardridge et al. (1981b). First, results obtained on parietal cortex (Chapman et al. 1977, Folbergrová et al. 1981) demonstrate a

linear relationship between blood and tissue glucose concentrations which is similar at 20, 60, and 120 min, and similar in "standard" and glucose-infused animals (Fig. 8).

Second, although the present results were

obtained on a small number of animals and although the data may be less accurate due to the dissection of multiple, small

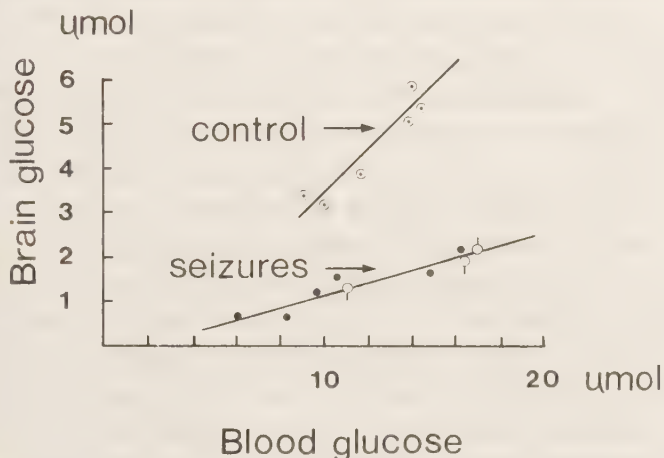


Figure 8. The brain tissue glucose as a function of whole blood glucose concentration in control and seizure animals. Circles with dot represent control animals and filled circles denote seizure animals (data from Folbergrová et al 1981). Unfilled circles represent mean values from Chapman et al (1977) and are drawn as means $\pm$ S.E. for groups of 20, 60, and 120 min of seizure activity.

tissue samples, the data showed that, in all "hypermetabolic" structures, the ratios obtained fell within the narrow range of 0.11–0.16. The results also demonstrate that two "hypometabolic" structures (cerebellum and inferior colliculus) consistently had ratios of 0.20, or higher. Finally, the data suggest that the ratio for thalamus may have increased somewhat when the seizures were prolonged from 20 to 120 min. In the case of hypothalamus, this clearly seemed to occur.

In order to allow an approximate correction of the lumped constant with the help of the Pardridge nomogram we arbitrarily assumed that all structures with a CMR_{gl} value of 175% of control, or higher (Table 4), had a tissue to whole blood glucose ratio of 0.14. Since the plasma to whole blood glucose concentration ratio (v/w) was found

to be 1.55 (mean of 24 determinations in control and seizure animals, $r=.99$) the corresponding ratio (tissue to plasma, w/v) for insertion into the Pardridge nomogram was 0.09. Thus, in each animal tissue glucose concentration was calculated from the plasma value, using this factor. On the basis of the results given in fig. 7 we further assumed that a lumped constant of 0.48 could be used for all structures with a normal or decreased metabolic rate.

Admittedly, the corrections made can give only approximate estimates of the true CMR_{gl} values, and it remains to be explained why $l-CMR_{gl}$ in cerebral cortical and limbic structures seems to increase when the seizure period is extended from 20 to 60 min. However, we submit that measurements on hyperglycemic animals and use of the Pardridge nomogram represent the best available approach to the effect of seizures on local metabolic rate. In support, we note that the $l-CMR_{gl}$ values for parietal and sensorimotor cortex, so derived, are close to the "cortical" $CMRO_2$ and CMR_{gl} values previously reported (Meldrum and Nilsson 1976, Borgström et al. 1976). Furthermore, inspection of the 20 $\mu\text{mol}\cdot\text{ml}^{-1}$ line in fig. 7 strongly indicates that any error introduced into the data of figs. 4-6 is so small that it will not affect the major conclusions drawn in the discussion to follow.

Local CBF

Horton et al. (1980) studied l -CBF during sustained bicuculline-induced seizures, using a ^{14}C -iodoantipyrine technique with tissue sampling rather than autoradiography, as well as a particle (microsphere) distribution method. The authors concluded that the latter method, which sometimes gave differences in flow rates between identical structures in the two hemispheres, systematically underestimated local CBF. Their ^{14}C -iodoantipyrine data indicated that, following an initial marked hyperemia, flow rates in all structures studied subsequently fell during the 2 hr seizure period (cf. Meldrum and Nilsson 1976) but, since CBF remained increased above control in selectively vulnerable areas (e.g. hippocampus), the authors concluded that a maintained intense seizure discharge must be mainly responsible for epileptic brain damage.

The resolution of the autoradiographic technique offers superior discrimination of local differences in blood flow during seizures. The present results demonstrate that in spite of the "generalized" nature

of the seizures induced, there were large differences in the l-CBF response. After 20 and 60 min, some regions showed only moderate increases in CBF while others increased their flow rates 3- to 4-fold. A comparison with control data (see Table 2 above) reveals that the increase in CBF bore no apparent relationship to the "resting" flow rates. The explanation for the local variability in CBF response is not readily apparent, especially since it did not correlate well with local metabolic rate (see below). Furthermore, the heterogenous increase in CBF cannot be related to differences in vasodilatory capacity between structures since hypercapnia induces a much more homogenous increase in local CBF (Dahlgren et al. 1981a and b).

Previous results on "cortical" CBF measured after 20, 60, and 120 min of bicuculline-induced seizures gave mean values of 4.1, 3.6, and 2.7 ml.g⁻¹.min⁻¹ (Meldrum and Nilsson 1976). If one assumes that the superior sagittal sinus, the sampling site for venous blood, predominantly drains frontal, parietal, and sensorimotor cortex, the present data are in reasonable agreement. Like Horton et al. (1980) we observed a decrease in l-CBF after 120 min, especially in cerebral cortical and some subcortical (e.g. limbic) structures. For example, after 120 min none of the cortical structures had flow rates greater than 1.7 times control, and some had CBF values close to control. In order to discuss the possible importance of this "delayed hypoperfusion" we must consider data on local CMR_{gl}.

Local CMR_{gl} and the l-CMR_{gl}/l-CBF couple

Results pertinent to these variables are illustrated in figs. 4-6 (see above). The data on relative changes in l-CMR_{gl} demonstrate two main features. The first relates to the fact that, in spite of the "generalized" nature of bicuculline-induced seizures, changes in l-CMR_{gl} were strikingly heterogenous. Three groups of structures could be discerned. In one, comprising the cerebral cortices and 3 of 4 limbic structures, CMR_{gl} exceeded 200% of control during at least 2 of the 3 periods studied, and in the limbic structures l-CMR_{gl} approached 400% of control. A second group was characterized by structures whose CMR_{gl} values were consistently below 200% of control, with some having values close to or below control (see especially cerebellum, inferior colliculus, superior olive, and habenula). It is tempting to conclude that such structures do not participate in the seizure discharge. However, it cannot be excluded that their metabolic

rate increased during the first 20 min. For example, results on tissue metabolites demonstrate a moderate perturbation of cerebellar metabolism at 20 min (Folbergrová et al. 1981, Siesjö et al. 1982). We also note from fig. 5 that at least three structures (thalamus, hypothalamus, and lateral geniculate body) consistently had $l\text{-CMR}_{gl}$ values in the 150-200% range, forming a group intermediate to those designated as "hypermetabolic" and "hypometabolic". The third group, finally, consisted of three functionally related structures whose $l\text{-CMR}_{gl}$ values changes continuously during seizures. Among these, the substantia nigra and globus pallidus had initially very high CMR_{gl} values that fell gradually to subnormal values, while the third (caudoputamen) showed continuously increasing values. In view of the fact that these structures mediate transmission in dopaminergic pathways it is of interest that Calderini et al. (1978) found increased tissue values of dopamine but evidence of reduced activity in dopaminergic neurons.

The second main feature of the CMR_{gl} results is the fact that CMR_{gl} in cerebral cortical and all limbic structures except habenula remained upheld at markedly increased values throughout the 2 hrs seizure period. Thus, it cannot be argued that the secondary reduction in CBF reflected metabolic depression.

Results on the coupling between local CMR_{gl} and CBF reveal a striking mismatch between metabolic rate and blood flow. On the one hand, although the cerebral cortices, 3 of 4 limbic structures, and the caudoputamen, initially showed proportional increases in CMR_{gl} and CBF, prolongation of the seizures to 120 min gave rise to a relative hypoperfusion due to maintained CMR_{gl} and reduced CBF. On the other hand, structures with low or intermediate increases in CMR_{gl} consistently showed a relative hyperemia with elevations in CBF that were out of proportion to the changes in CMR_{gl} . Striking examples were provided by the colliculi, superior olive, and habenula, but it should also be recalled that, at 120 min, $l\text{-CMR}_{gl}$ in the substantia nigra was reduced to <20% of control at a CBF of 150% of control. Clearly, seizures represent a condition during which metabolic rate and blood flow may be grossly uncoupled, for reasons that are presently not known.

In the introduction we emphasized that measurements of local metabolic rate and blood flow may yield information on the mechanisms

that contribute to give neuronal cell damage. Current results suggest that, at least in ventilated animals, such damage is confined to cerebral cortical and limbic strictures. We note that these structures are those in which metabolic rate is markedly enhanced, and which develop a relative hypoperfusion. It remains to be shown whether the development of cell damage relates to the enhanced metabolic rate, with the reduction in CBF occurring as a secondary phenomenon of little pathogenetic importance, or if the relative hypoperfusion contributes to the damage incurred.

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